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INFECTIOUS ABORTION OF CATTLE

AND THE OCCURRENCE OF ITS BACTERIUM IN MILK.

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INFECTIOUS ABORTION OF CATTLE AND THE OCCURRENCE OF ITS BACTERIUM IN MILK.

I.—INTRODUCTORY STATEMENT.

By A. D. MELVIN,
Chief of the Bureau of Animal Industry.

In a paper presented at the annual meeting of the American Veterinary Medical Association on August 24, 1911, by Drs. E. C. Schroeder and W. E. Cotton, of the Experiment Station of the bureau, under the title "An Undescribed Pathogenic Bacterium in Milk," attention was called to a peculiar pathological condition frequently found in guinea pigs that were injected with milk and afterwards permitted to live six weeks or longer.[1] This paper under a different caption and with slight modification appears in the following pages.

The microorganism to which the pathological condition in the guinea pigs was due was isolated, and it was definitely proven that it, and it alone, was the cause of the lesions, which, in their gross anatomical appearance, resembled the lesions of tuberculosis in guinea pigs so much that a distinction between the two pathological conditions was not certainly possible without microscopic examination. The microorganism was studied as carefully as the facilities for bacteriological work at the Experiment Station permitted.

As a matter of course, the discovery of the peculiar microorganism, a small bacterium, aroused considerable interest in the bureau, and it was not long before Dr. John R. Mohler, Chief of the Pathological Division, called attention to the close resemblance of the organism found in milk to the bacterium of infectious abortion, in which latter he was interested because he was making investigations concerning contagious abortion of cattle.

Experiments were at once undertaken, by Mohler on one hand and by Schroeder and Cotton on the other, to settle definitely the question of the identity of the two bacteria. In February, 1912, the experiments reached the stage where it was absolutely certain that the bacillus isolated from milk—the organism that causes a slowly progressive disease in guinea pigs with lesions macroscopically like those of tuberculosis—is identical with the bacillus of infectious abortion of cattle. A preliminary note was therefore issued March 2, 1912, as Circular 198 of the Bureau of Animal Industry, announcing

[1] See Proceedings of the American Veterinary Medical Association, 1911, pp. 442–449. Also American Veterinary Review, Nov. 11, 1911, pp. 195–206.

that the bureau's investigations had demonstrated the identity of the bacterium discovered in the milk of cows and the organism widely recognized as the etiological factor in infectious abortion of cattle.

Those who are familiar with the descriptions that have been given of the organism of infectious abortion of cattle will easily recall how contradictory they are in many respects. Whether or not this is due to differences in the technique used by different investigators can not be said, but it is interesting to refer to the matter here, because the identity of the bacillus in milk and the organism of infectious abortion of cattle studied by Mohler would have been decided much earlier if one difficulty, now settled, had not stood in the way, namely, that the tests made at the Experiment Station at first showed the bacillus from milk to be a Gram positive organism while the other bacillus was found by the Pathological Division to be Gram negative. The bacillus, whether obtained from milk or from other sources, is now looked upon as being Gram negative.

The final test to establish the identity of the bacilli was the similarity obtained by Mohler and Traum in the Pathological Division and by Schroeder and Cotton at the Experiment Station, working independently, in the lesions produced in guinea pigs inoculated respectively with the milk bacillus and the bacillus of infectious abortion, as well as the identity of the two organisms shown through the agency of the complement-fixation test.

In what respect the discovery of the bacillus of infectious abortion of cattle in the milk of cows is important can readily be seen by reading the paper by Schroeder and Cotton, which follows. In that paper it is pointed out that it is questionable what may be the practical importance of this bacillus, now demonstrated to be the bacillus of infectious abortion, occurring with a high degree of frequency in market milk. The fact that this organism was found in 8 samples of market milk among 77 samples tested (over 11 per cent), and in the milk distributed by 6 among 31 dairies (over 19 per cent), leaves no doubt that we are dealing with a phenomenon that is ominously serious in its significance for public health.

We do know that the organism causes abortion in cattle and some other animals, and that it causes progressive lesions of a serious character in some experiment animals; and although we do not know what effect it may have on human beings, we can not afford to assume that they are not affected by it. This bacillus may prove to be another source of danger in the use of raw milk as food, and may furnish an additional reason for taking advantage of the safeguard afforded by pasteurization.

The paper by Schroeder and Cotton follows, after which is given a paper by Mohler and Traum reporting the work done in the Pathological Division.

II.—THE BACILLUS OF INFECTIOUS ABORTION FOUND IN MILK.

By E. C. SCHROEDER, M. D. V., and W. E. COTTON, D. V. M.,
Of the Bureau of Animal Industry Experiment Station, Bethesda, Md.

CIRCUMSTANCES LEADING TO DISCOVERY OF THE ORGANISM.

Several years ago, at the Experiment Station of the Bureau of Animal Industry at Bethesda, Md., we made a series of tests relative to the occurrence of virulent tubercle bacilli in ordinary city milk, with special reference to their intermittent occurrence in milk vended by dairies from which tuberculous samples had been obtained with previous tests. In the course of these investigations many guinea pigs were given intraabdominal injections of milk and afterwards kept alive somewhat longer than is commonly believed to be necessary for well-marked lesions of tuberculosis to develop. When the guinea pigs were eventually killed some of them showed lesions on post-mortem examination that could easily be mistaken for tuberculosis, but which our experience with tuberculosis in guinea pigs helped us to distinguish as probably another disease, especially as careful microscopic examinations failed to reveal acid-fast bacilli, which are, as a rule, abundant and not difficult to find in the tuberculous lesions of guinea pigs.

We soon discovered the disease to be transmissible through subcutaneous inoculations of affected tissue from guinea pig to guinea pig, but our efforts to cultivate a supposedly existing specific microorganism and our attempt to find an organism in the lesions under the microscope were unsuccessful.

After our interest in the subject had somewhat abated it was actively restimulated during a study concerning the influence of tuberculin injections on the elimination of tubercle bacilli, with milk and otherwise, from the bodies of tuberculous cattle, by discovering that the milk of a tuberculous cow at the Experiment Station caused the mysterious disease when it was injected into guinea pigs that were afterwards permitted to live six weeks or longer. We collected milk from this cow repeatedly under the strictest conditions to exclude its infection from any source but the interior of her udder, and this milk proved to be fully as infectious as that collected earlier with less minutely elaborate precautions.

We again tried to isolate a specific microorganism, and as no growth appeared in the numerous tubes of culture media inoculated with small fragments of tissue from affected guinea pigs, we assumed that we were dealing with an organism that either could not be cultivated artificially or that would not grow in the culture media we had used; hence, as the disease was particularly severe in its action on the livers of guinea pigs, we concluded to try a culture medium to which bile had been added.

ARTIFICIAL CULTIVATION OF THE BACILLUS.

On agar containing 6 per cent of glycerin and from 1 to 20 per cent of ox gall a growth was obtained which was more vigorous in the tubes to which 5 per cent or more of gall had been added than in those that contained less. Since obtaining this growth we have succeeded in cultivating the organism, which is a small bacterium, on other media, and have found that on some, on which it did not seem to grow at first, it multiplies quite well when the surface is smeared with pulp from the spleens of healthy guinea pigs.

We are not yet fully prepared to make a detailed statement about the different substances on which the germ will grow, especially as it multiplies better on several media after it has lived one or more generations under artificial conditions. Its appearance on the surface of glycerin-bile-agar is in the form of small, pearly, slightly convex, pale-gray colonies. The water of condensation in slanted tubes remains clear, but is covered with a very thin, broken, almost imperceptible layer that looks like a small amount of fine, white dust deposited on the surface of a fluid which does not wet it and into which it can not sink. A similar thin dustlike layer, beneath which the fluid remains perfectly clear, forms on the surface of some liquid media. This dustlike layer, when smear preparations of it are made and examined microscopically, has the appearance of a pure culture of the germ. In stab cultures the growth is located mainly at the surface, about the point of the puncture, giving the impression that multiplication, as with the tubercle bacillus, depends upon actual contact with air.

No growth has been obtained on any gelatin medium. On potato an almost imperceptible, flat, glistening layer with a very faint pink hue is formed. We are not certain that growth occurs in milk. Milk tubes inoculated directly from glycerin-bile-agar tubes and milk tubes inoculated from such milk tubes contain enough infection to cause the disease on injection into guinea pigs; but when the transfers from milk tube to milk tube are carried to the sixth generation the fluid seems to be innocuous and no germs are distinguishable microscopically in any of the milk tubes. On all media the growth is slow.

The temperature required for artificial cultivation is, as far as we have been able to determine, from 37° to 39° C., and the thermal death point of the bacterium is 60° C., maintained for 15 minutes.

DESCRIPTION OF THE BACILLUS.

The germ, which has now been definitely identified as the *Bacillus abortus*, was at first believed by the writers to be Gram positive, but is now known to be Gram negative. It is a nonacid-fast bacillus, with rounded ends, about the size of a tubercle bacillus of the bovine type. On cover glasses from cultures, stained with Loeffler's methylene blue, the individual bacilli appear very minute and somewhat separated from each other; stained with Stirling's or anilin gentian-violet they appear to be larger and to lie closer together.

INOCULATION TESTS.

We have repeatedly isolated this bacillus from the lesions in affected guinea pigs, have grown it in pure cultures, have caused the disease in other guinea pigs with the pure cultures, and have recovered pure cultures from the tissues of the latter. So far the guinea pig is the only animal species for which we have found it to be pathogenic, although we have injected it into rabbits, hogs, sheep, cats, dogs, chickens, and cattle. Its extremely slow and chronic action on guinea pigs, however, suggests that further inoculation tests with the other species of animals, if a sufficiently long period of time is permitted to pass, may give positive results.

LESIONS CAUSED IN GUINEA PIGS.

Guinea pigs become infected either through the inoculation or the ingestion of pure cultures or of naturally infected milk, but show no well-marked lesions until after the passage of six weeks or more. The gross anatomical lesions are an extreme enlargement and edema of the lymph glands generally; the appearance of small glistening nodules in the lungs, which seem to be caused by the enlargement of minute lymph glands that are ordinarily too small to be visible; the conversion of the minute nodules in the lungs into larger, necrotic areas; an enormous enlargement of the spleen, often to thirty or forty times its normal volume; an irregular thickening of the capsule of the spleen, through which its surface becomes marked with white areas varying in size from mere points to several centimeters in diameter; an enlargement and degeneration of the liver, which organ becomes thickly beset on surface and section with irregular, pale yellow or dirty white areas that seem to be due to an enormous proliferation of connective tissue and a consequent crowding out and obliteration of the liver cells proper; a diffuse, parenchymatous nephritis that

reaches stages in which dense, fibrous nodules are formed in the cortex of the kidneys; and, in male guinea pigs, a degeneration of the testicles, commonly beginning in the epididymis and often resulting in the conversion of one or both testicles into structureless cysts filled with creamy pus. When the disease is due to subcutaneous inoculation of pure cultures, there are no local lesions or pathological conditions referable to the point at which the inoculation was made. The lesions produced by this bacillus are shown in Plates XXIII and XXIV, and for comparison the lesions caused by the tubercle bacillus are shown in Plate XXV.

In a small but not inconsiderable proportion of the infected guinea pigs a curious enlargement about some of the bone articulations occurs, through which the affected joint becomes stiff and useless. This condition is especially interesting from the bacteriological point of view, because, in the several cases in which careful examinations were made, it was found to be associated with a fairly large micrococcus, thus forecasting the possibility (as the condition has never occurred among the numerous other guinea pigs we have had under observation at the Experiment Station) of a microorganism that is harmless by itself but capable of doing serious injury in symbiosis with another microorganism. That this is not altogether a hypothetical view, but rather an inference drawn from experimental though as yet admittedly inadequate evidence, is shown by the occurrence of the joint disease in 1 of 6 guinea pigs, 2 of which were injected with a pure culture of the bacillus, 2 with a pure culture of the micrococcus, and 2 with mixed cultures of the bacillus and the micrococcus. It was 1 of the latter 2 that became affected with the joint disease. The 2 guinea pigs injected with pure culture of the micrococcus remained perfectly healthy, and the 4, of which 2 received pure culture of the bacillus and 2 mixed cultures of the two bacteria, in addition to the joint disease in 1, all developed the characteristic lesions the bacillus causes in guinea pigs.

OCCURRENCE OF THE BACILLUS IN MILK.

Probably the most remarkable thing about the bacillus is its expulsion from the bodies of apparently healthy cows with their milk, and hence it is desirable to show that this is really a fact and not a supposition backed by doubtful evidence. First, the bacillus was repeatedly proved to occur in milk, collected with the utmost precautions against extraneous contamination, from a number of cows that had previously been found to be infected; and, second, its presence in the milk and in tissue from the udder and supramammary lymph gland of one cow was proved in the following manner:

Station cow No. 220, which had been known for some time through the injection of her milk into guinea pigs to be infected, was killed.

Immediately before her death her udder was carefully washed and disinfected and her teats closed with strong ligatures, and directly after her death her udder, including the supramammary lymph glands, was cut from her body. The skin was then dissected from the udder and the entire denuded surface thoroughly scorched with the flame of a large Bunsen burner; the flamed surface was next incised with a sterile knife and milk collected in sterile pipettes, through the incisions, separately from each quarter, at points well removed from the teats. With equal precautions fragments of tissue were taken from one front and one hind quarter of the udder, from the supramammary lymph gland, and from the liver and the spleen; and this material was inoculated subcutaneously into 15 guinea pigs, as follows:

One guinea pig, milk from right front quarter of udder; result positive.
One guinea pig, milk from right hind quarter of udder; result negative.
One guinea pig, milk from left front quarter of udder; result positive.
Two guinea pigs, milk from left hind quarter of udder; result negative.
Two guinea pigs, tissue from front quarter of udder; result positive.
Two guinea pigs, tissue from hind quarter of udder; result negative.
Two guinea pigs, tissue from supramammary lymph gland; result positive.
Two guinea pigs, tissue from spleen; result negative.
Two guinea pigs, tissue from liver; result negative.

The quantity of milk obtainable from the cow's udder, as she was nearly dry at the time of her death, was very small; otherwise two guinea pigs would have been inoculated with milk from each quarter.

It should be noted that the inoculations show positive results with both the milk and the tissue from the front quarters of the udder and negative results with both the milk and the tissue from the hind quarters, and that strength is added to the evidence which proves that the germ was located in the depths of the udder by the positive results obtained with the inoculation of tissue from the supramammary lymph gland.

The post-mortem examination of the cow revealed nothing to explain the persistent occurrence of the bacillus in her milk, excepting a few small areas of slight induration in her udder. A second cow that also expelled the bacillus with her milk has been killed, examined post-mortem, and material obtained from her body for guinea-pig injections. The autopsy showed no other lesions than were found in the first cow, and it is too early to report on the guinea pigs. It may be well to add that the blood and urine of several cows from which infected milk was obtained were tested by inoculating guinea pigs, and invariably failed to cause disease.

Some studies relative to the way in which the udders of cows become infected have been made. Several cows were selected and proved by repeated injections of their milk into the abdominal cavities of guinea pigs to be free from the infection. On the udder

and teats of one cow pure cultures of the bacillus were rubbed; another cow was fed pure cultures in her drinking water, and a third was given subcutaneous injections of pure cultures. This work is not yet complete, but as far as it has gone it has given negative results.

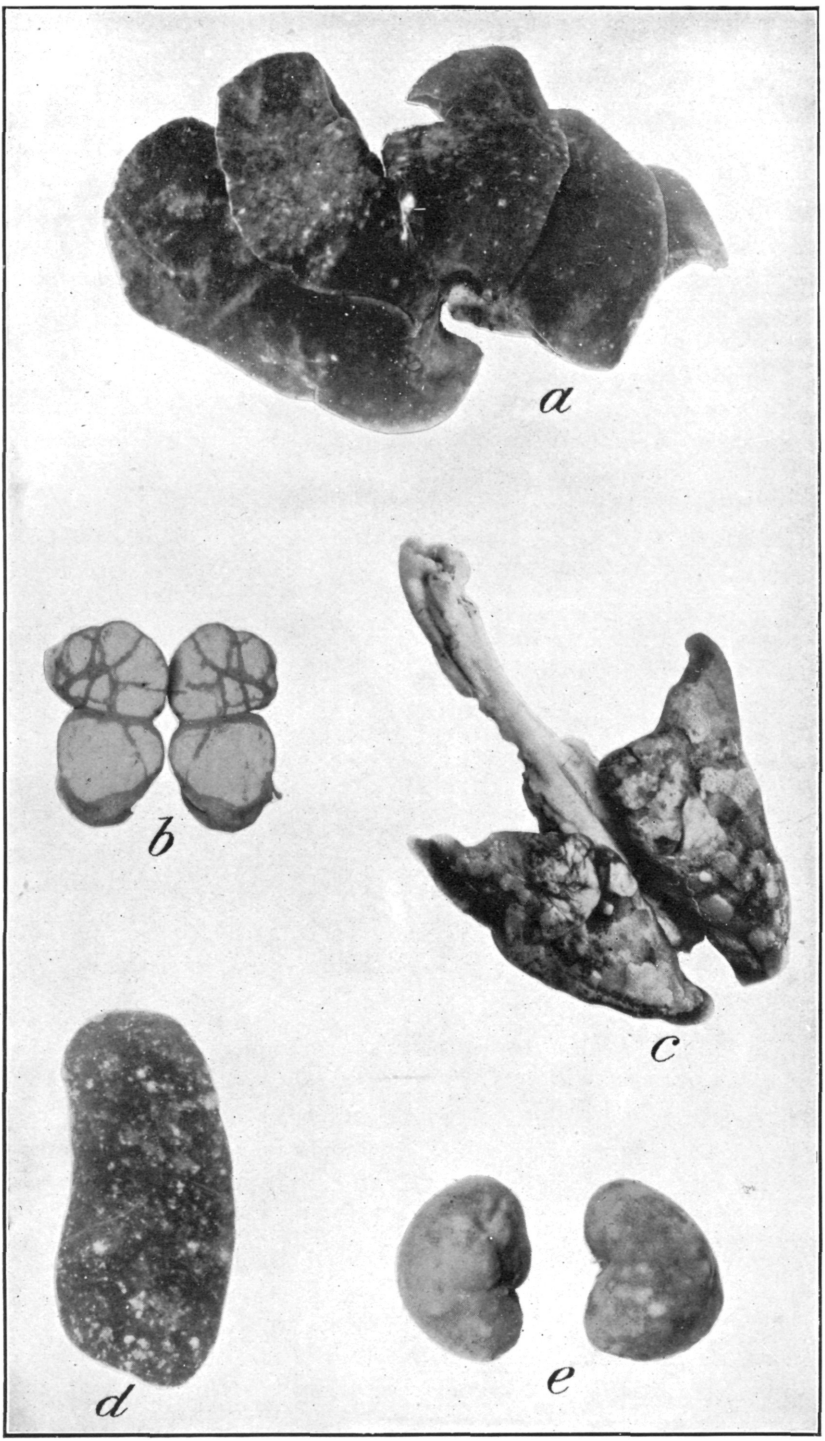
The frequency with which we found the bacillus to occur in ordinary market milk is illustrated by the samples examined since we first observed the curious disease in guinea pigs. In one series we tested 77 samples of milk from 31 dairies, and 8 of the samples derived from 6 different dairies were found to be infected. In another series we tested 140 samples from 4 dairies, with the following results: Dairy A, 35 samples, 11 infected; dairy B, 33 samples, 7 infected; dairy C, 34 samples, 2 infected; dairy D, 38 samples, 2 infected. The 77 samples in the first series of tests, with the 140 in the second, make a total of 217, among which 30, or nearly 14 per cent, proved to contain the bacillus. As dairies A, B, C, and D are among the 31 from which the 77 samples of the first series of tests were obtained, and are 4 of the 6 infected, the proportion of infected dairies is not changed by the second series of tests.

In addition to the samples of market milk we have tested one sample each of the milk from 140 dairy cows that constitute a single large herd in the District of Columbia, and from 36 cows that belonged to the Experiment Station at Bethesda, Md. Among the 140 cows the bacillus was found in the milk of 19, and among the 36 cows it was being passed by 11. The 140 cows form a herd that has been repeatedly tested with tuberculin and is very probably free from tuberculosis. A large proportion of the 36 station cows were affected with advanced tuberculosis, and among the 11 that were found to be passing the bacillus 8 were advanced cases of tuberculosis, 1 was affected with actinomycosis, and 2 were apparently healthy.

One of the tuberculous cows at the station that was passing the bacillus was affected with tuberculous disease of her udder. Her milk caused both tuberculosis and the other disease in the guinea pigs injected with it, showing that the two diseases can live in harmony in one animal body; in fact, each seemed to increase the pernicious potency of the other. (See Pl. XXVI.) Guinea pigs with the double infection formed interesting subjects, because the tubercle bacillus in the lesions could easily be demonstrated in stained preparations, while the other bacillus, which was microscopically invisible to us, could be cultivated on media that is unsuitable for the tubercle bacillus.

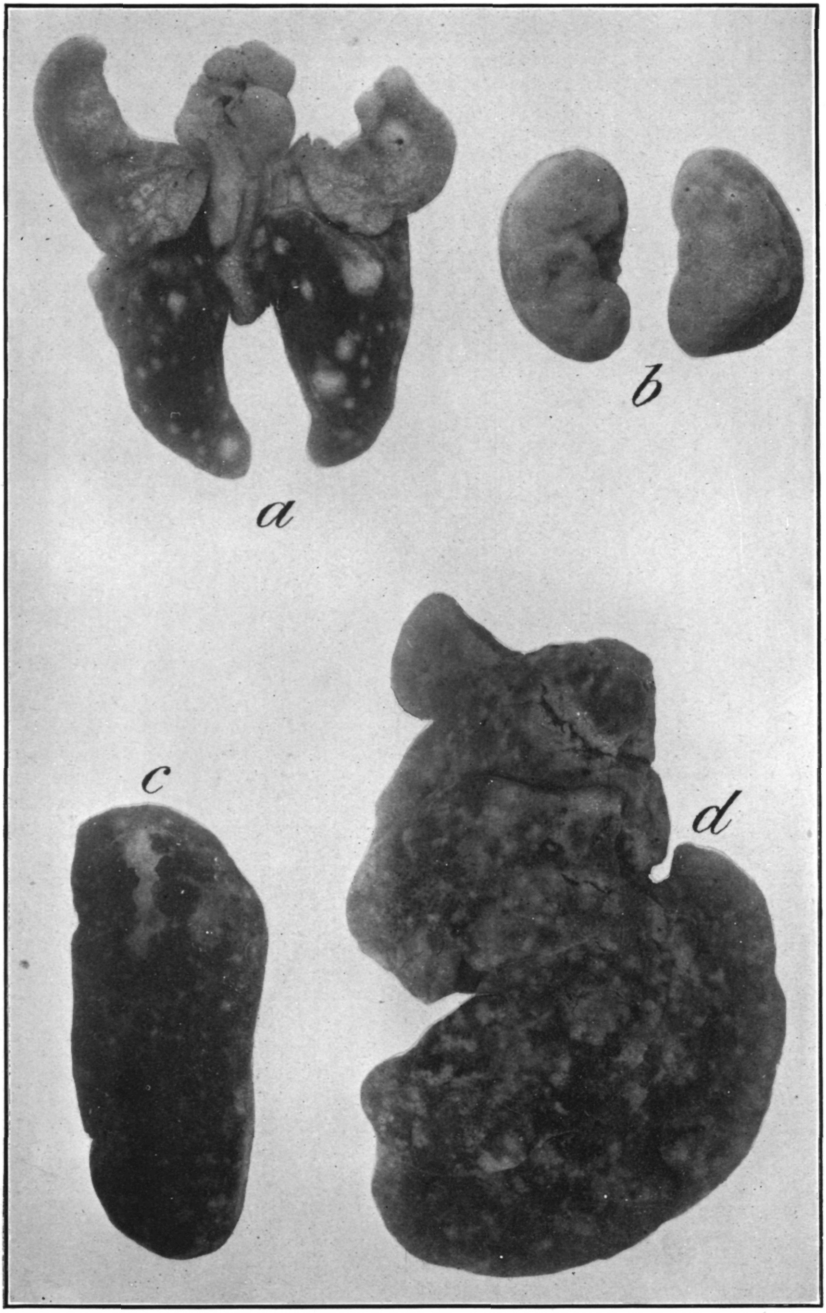
CONCLUSION.

What the real significance or practical importance of this bacillus, the presence of which in milk appears to have escaped detection in the past very likely because of the difficulties associated with



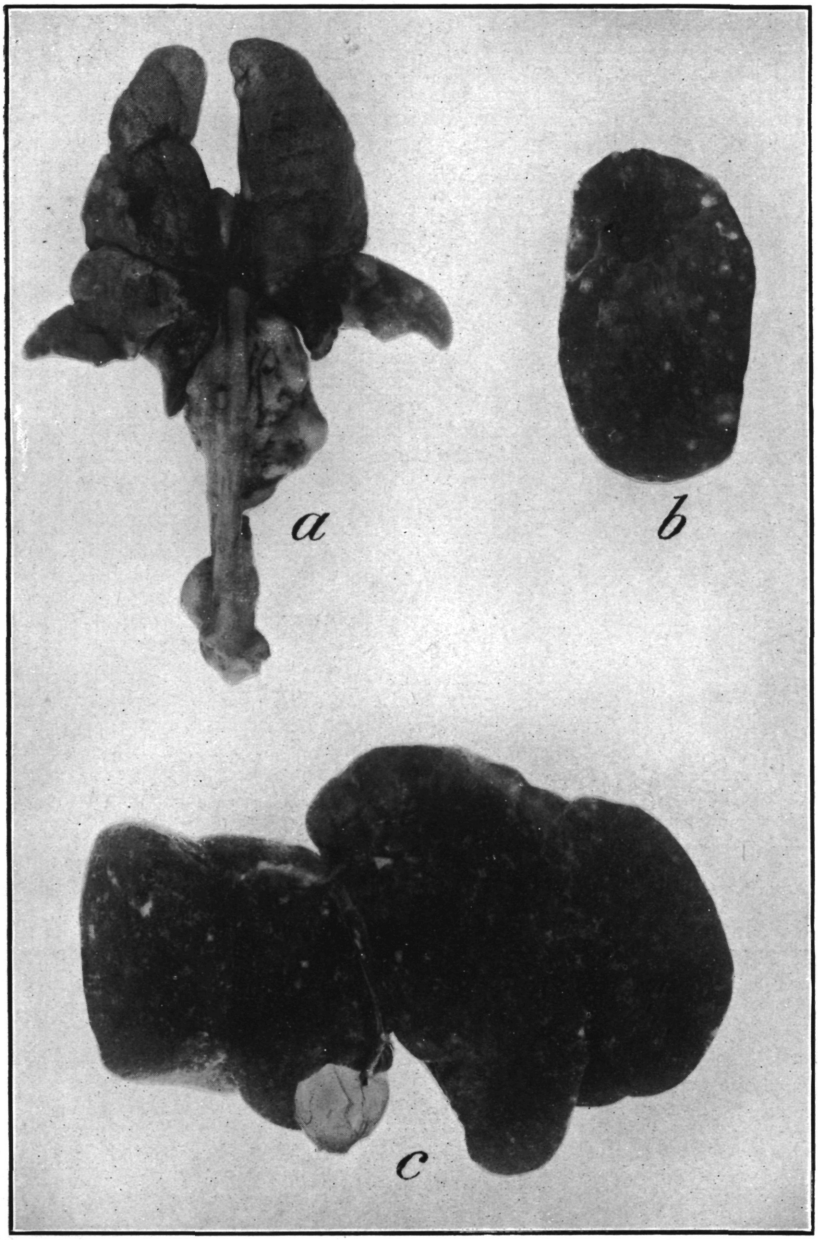
ORGANS OF GUINEA PIG, SHOWING LESIONS CAUSED BY ABORTION BACILLUS.

a, Liver; *b*, testicles; *c*, lungs; *d*, spleen; *e*, kidneys. The white spots in the kidneys are fibrous nodules in the degenerated cortex. The testicles are necrotic throughout, excepting the dark lines, which are bands of connective tissue.



ORGANS OF GUINEA PIG, SHOWING LESIONS CAUSED BY ABORTION BACILLUS.

a, Lungs; *b*, kidneys; *c*, spleen; *d*, liver. The white spots in the lungs are an advanced stage of a condition which first manifests itself in the form of very minute, glistening, almost transparent nodules, which gradually increase in size, develop gray centers, and finally become comparatively large necrotic areas.



ORGANS OF GUINEA PIG, SHOWING LESIONS CAUSED BY TUBERCLE BACILLUS.

a, Lungs; *b*, spleen; *c*, liver. A comparison of this plate with Plates XXIII and XXIV will show the remarkable similarity in the macroscopic appearance of the lesions caused in guinea pigs by the abortion bacillus and by the tubercle bacillus.

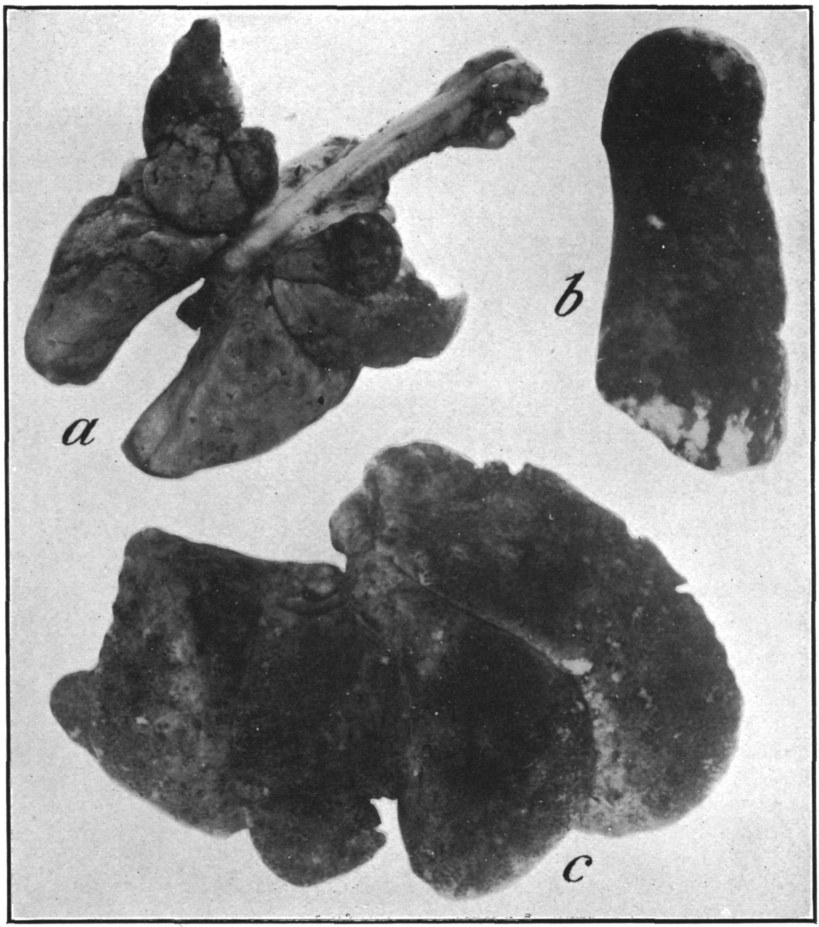


FIG. 1.—ORGANS OF GUINEA PIG, SHOWING LESIONS CAUSED BY ABORTION BACILLUS IN COMBINATION WITH TUBERCLE BACILLUS.

a, Lungs; *b*, spleen; *c*, liver. Each bacillus seems to increase the pathogenic potency of the other for guinea pigs.

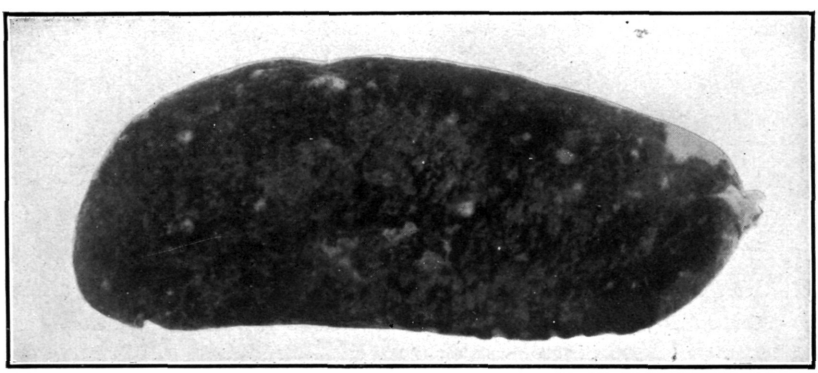


FIG. 2.—SPLEEN OF GUINEA PIG AFFECTED WITH TUBERCULOSIS IN COMBINATION WITH DISEASE CAUSED BY ABORTION BACILLUS.

Weight of spleen, 31 grams. The normal weight of a guinea-pig spleen is less than 1 gram. The organ weighed more than 40 times as much as a normal spleen, its enormous size being due to the abortion bacillus.

its artificial cultivation and the length of time it requires to cause well-marked lesions in guinea pigs, may ultimately prove to be we are unable to say. But no one can doubt that the common occurrence of a microorganism, pathogenic for any species of animal, in an article of food as widely and as extensively used as milk, deserves that we should study it with the greatest care, especially after it has been shown that it is an organism which has the udders of apparently healthy cows as its normal habitat, and which therefore can not be certainly excluded from milk, no matter how much cleanliness and care are used in its production. In this sense the germ forms another link in the long chain of facts that point unmistakably to the proper pasteurization of all milk before it is used as food as a measure essentially necessary for the protection of public health.

ADDENDA.

The data available at the Experiment Station of the bureau show conclusively that the bacillus with which the foregoing paper deals, and which is now known to be the bacillus of contagious abortion of cattle, may be eliminated continuously for years in the milk of infected cows that no longer abort. The following history of two cows illustrates this remarkable fact:

Cow 637.

June 15, 1908, received at the Experiment Station, age about 5 years.
October 15, 1908, gave birth to a healthy female calf.
February 29, 1909, test of milk proved that it contained the bacillus now known to be the germ of infectious abortion.
May 18, 1909, bacillus proved to be in milk.
May 24, 1909, bacillus proved to be in milk.
September 14, 1909, gave birth to a healthy female calf.
October 14, 1909, bacillus proved to be present in milk from all four quarters of udder.
February 14, 1910, milk tested and proved to be infected.
September 22, 1910, milk tested and proved to be infected.
November 3, 1910, milk tested and proved to be infected.
November 18, 1910, milk tested and proved to be infected.
November 23, 1910, milk tested and proved to be infected.
July 26, 1911, aborted, 5 months fetus. Tests proved the bacillus to be present in the abdominal fluid, liver, and spleen of the fetus.
July 31, 1911, milk tested and proved to be infected.
September 22, 1911, milk tested and proved to be infected.
October 2, 1911, butter made from milk of this cow found to be infected.
October 9, 1911, milk tested and proved to be infected.
December 4, 1911, milk tested and proved to be infected.

Later tests have been made, but the guinea pigs inoculated with the milk of the cow have not yet been autopsied.

The record shows that the bacillus was present in the milk of the cow from February 29, 1909, to December 4, 1911, or a period of

about two years and nine months. There is no reason now, after three years, to believe that it has disappeared from the milk. Whether this cow ever aborted before she was received at the station is not known. It is remarkable that she produced a healthy calf about six and one-half months after the abortion bacillus was found in her milk, and then aborted at her next pregnancy, two years and five months after her milk was known to be infected. The milk caused the peculiar tuberculosislike lesions in guinea pigs both through inoculation and ingestion.

Cow No. 642.

June 15, 1908, received at the Experiment Station, about 6 years old.
June 20, 1908, aborted; precise age of fetus not known. The abortion was attributed to something incident to transportation from place of former owner to the station.
December 18, 1909, gave birth to a healthy male calf.
October 24, 1910, aborted, 7 months fetus. Tests of fetus showed the presence of the bacillus.
November 21, 1910, milk tested and proved to be infected.
December 1, 1910, milk tested and proved to be infected.
May 22, 1910, milk tested and proved to be infected.
February 23, 1911, milk tested and proved to be infected.
September 11, 1911, milk tested and proved to be infected.
October 9, 1911, gave birth to a healthy female calf.

The record shows that the milk was infected from October 24, 1910, to September 11, 1911. Later tests will probably show that the milk is still infected.

III.—INFECTIOUS ABORTION OF CATTLE.

By JOHN R. MOHLER, V. M. D., *Chief of the Pathological Division,*
AND
JACOB TRAUM, D. V. M., *Veterinary Inspector, Pathological Division.*

INTRODUCTION.

In reviewing the field of veterinary research covered during the year 1911 the particular event which indicates marked progress in the study of animal diseases is the work on infectious abortion that is being conducted in this and other countries.

From the viewpoint of economic importance, infectious abortion of cattle ranks second only to tuberculosis, and in certain sections of the country even supersedes the latter in the monetary loss it occasions. Aside from the loss of the calf, the loss occasioned by the reduction in milk supply, together with the failure to conceive for several months or forever after the abortion, and the frequency of retained placenta, has made this disease the bane of dairymen and stock raisers.

The exact financial loss can not be even approximately estimated, but from the fact that the disease exists in all sections of the country, both in dairy and range cattle, as is evidenced by the reports from various State officials, and from the inquiries received at this bureau regarding this disease, it can safely be stated that the direct loss reaches into the millions, while the potential loss is likewise enormous and inestimable. Thus in the last two years the Pathological Division of this bureau received correspondence from 32 different States regarding treatment, prevention, and eradication of the disease. Furthermore, the disease is of such an insidious character that it may be brought into a herd by an unsuspected animal without attracting attention, inasmuch as there are no readily noted symptoms present in the diseased animal.

In this publication we will concern ourselves only with the type of abortion as found in enzootic or epizootic form among cattle. We will not include the premature delivery of the fetus caused by mechanical injuries, frozen, moldy, or smutty feed, or by other causes of a noninfectious character, nor will we refer to those infectious diseases affecting the mother during the course of which the fetus is expelled from the uterus prior to the completion of the full period

of gestation. Instead we will limit ourselves to that form of abortion in which there is practically no apparent change in the health of the mother prior to the abortion.

DEFINITION.

Infectious abortion of cattle is a specific infectious disease produced by the *Bacillus abortus* of Bang and characterized by inflammatory changes of the mucous membrane of the uterus and fetal membranes, resulting as a rule in the premature expulsion of the fetus.

Synonyms: Contagious abortion; epizootic abortion; enzootic abortion; slinking of calves.

HISTORY.

This disease has been known in England and Continental Europe for many years, and descriptions of it are mentioned in the writings of Mascal, Lafoose, Skellet, Lawrence, St. Cyr, Zündel, and Youatt. In the early part of the eighteenth century British veterinarians recognized its contagiousness, but it remained for Franck (1876), Lehnert (1878), and Bräuer (1880) to produce the disease in healthy pregnant cows by the introduction of exudate and material from aborting animals. Nocard (1888) isolated from the exudate between the mucous membrane of the uterus and fetal membranes a micrococcus and a short bacillus which were found continually in contagious abortion, but he failed to reproduce the disease by inoculations of pure cultures of these organisms into healthy pregnant animals. In 1897 Bang,[a 1] assisted by Stribolt, published their findings regarding infectious abortion of cattle, in which they incriminated as the causative agent Bang's bacillus of abortion. With pure cultures of this bacillus they were able to produce the disease artificially and to recover the same organism from the experimental cases.

Preisz,[21] Nowak,[20] Zwick,[28] Holth,[10] McFadyean and Stockman,[15] MacNeal and Kerr,[14] Good,[7] Giltner,[6] and others have corroborated these findings, especially with reference to the causative agent. Grinsted,[8] Wall,[24] Holth,[9] Brüll,[4] Larson,[12] and other workers have more recently concerned themselves principally with the diagnosis of the disease.

CAUSE.

Bang and Stribolt obtained in pure culture a short, nonmotile, Gram negative, pleomorphic rod from the exudate found between the mucous membrane of the uterus and the fetal membranes of a cow which was killed while showing the symptoms of approaching abortion. These investigators proved that the organism was the

[a] Superior numerals refer to bibliography at end of paper.

specific cause of abortion by injecting pure cultures of this bacterium into the vaginas of two cows and producing abortion in one after a little over two months. The other cow was killed while showing premonitory symptoms of the disease. In both instances they recovered the *Bacillus abortus* from the exudate of the uterus. These investigators were also successful in producing abortion in pregnant cows by intravenous injection and the feeding of pure cultures of the organism, and in every instance recovered the organism from the experiment animals. Their findings have since been substantiated by many European observers and particularly by McFadyean and Stockman, of England.

In this country Moore [13] and Law, in New York, and Chester, in Delaware, incriminated a variety of the colon bacillus as the causative agent. MacNeal and Kerr [14] were the first American workers, however, to record the isolation and cultivation of Bang's bacillus of abortion from the placentas of two clinical cases of infectious abortion. These workers used the Nowak plate method for obtaining this organism in pure culture. They also succeeded in growing the organism on the slanted surface of serum agar by inoculations from plate colonies, but only in the presence of the *Bacillus subtilis*. There is no doubt that the *Bacillus abortus* has been observed by several American workers during the past decade, but in view of the fact that the organism found by them could not satisfy the specific biological requirements as set forth by Bang, and especially so with reference to the oxygen optima, confirmatory results were not published. Thus it was the accepted opinion that colonies develop only in a zone about a centimeter from the surface of a shake culture, extending to 2 to 2½ centimeters from the surface, Bang claiming that this organism was neither aerobic nor anaerobic. He also asserted that the oxygen optima was, first, a degree of oxygen tension in nutritive medium less than that of atmospheric air, and, second, the presence in the nutritive medium of a very high oxygen tension, which, however, lies somewhat under 100 per cent. He claimed that between these two optima there is an intermediate zone in which the abortion bacillus grows badly or not at all.

The English commission [15] found that an organism isolated from the uterine exudate of a typical case of infectious abortion would grow readily in the presence of air, and in several instances the growth appeared on the surface of the water of condensation before it did in the subsurface. In view of Bang's biologic requirements they at times doubted that they were dealing with Bang's bacillus of abortion, and in several instances resorted to inoculations and comparisons with cultures obtained from Bang, with the result that the identity of the organisms was proved conclusively.

In the Pathological Division of the Bureau of Animal Industry it was found by the writers that the organisms, obtained from the stomach of an aborted fetus and grown according to Bang in agar-gelatin horse-serum shake cultures, would appear as small bluish-green colonies on the side of the tube in the presence of air, and with several reinoculations on slant agar, with or without glycerin, luxuriant growths could be obtained. The growth on the slant agar is as a rule rather slow at first, but after several generations under aerobic conditions an excellent growth can be obtained within 1 to 3 days.

The organisms isolated by us agree in morphology, cultural characteristics, and staining properties with those described by the Danish, English, and other observers, and pure cultures of the bacillus are capable of producing abortion in pregnant animals, from the tissues of which the organism may be readily recovered. Even with these requirements satisfied it has been the practice in this laboratory to make both an agglutinating fluid and an antigen with these organisms and test them with a known serum before identifying them as abortion bacilli.

DESCRIPTION AND CHARACTERISTICS OF THE CAUSATIVE ORGANISM.

MORPHOLOGY.

Bacillus abortus is a short, nonmotile, pleomorphic rod, most often of a coccoid form, 0.4 to 0.6 μ wide by 1 to 2 μ long; frequently its length will be less than 1 μ or as long as 3 μ. A smear from the exudate or gastro-intestinal contents of the fetus will show the bacilli in masses composed of short coccoid or longer forms. Cultures, especially young ones, as Holth describes, show more regularity and usually are of distinct bacillary form. Involution forms of various shapes are observed in smears from older cultures. (Pl. XXVII.)

STAINING.

Ordinary anilin dyes stain the organism satisfactorily. In some smears the bacilli do not take the stain as readily as in others. Carbol fuchsin followed by 1 per cent acetic acid and also Loeffler's methylene blue bring out the pleomorphism of this organism to advantage. Unstained areas will be seen in the center, giving the bacillus the appearance of a polar or peripheral stained organism. Other bacilli will show clear or lighter areas on the sides or at the poles. The more intense staining reagents, as thionin, carbol fuchsin, gentian, or methyl violet, will stain the organism uniformly. Dilute carbol fuchsin, recommended by the English commission, gives very desirable results. In sections of tissues methyl green pyronin has given

fairly satisfactory results. With the Gram method of staining negative results are always obtained. In a few instances, however, the organism may retain a very slight amount of stain as compared with Gram positive organisms used as controls on the same slide. (Pl. XXVII.)

CULTURAL CHARACTERISTICS.

Relative to culture media, it was found that the agar gelatin raw-serum shake of Bang and the autoclaved alkalinized agar gelatin serum of the English commission both gave satisfactory results in obtaining cultures from the uterine or fetal material in a good state of preservation. Still more satisfactory was an agar gelatin serum mixture, the serum in this case being heated in Arnold's sterilizer at 60° C. for one hour each day for four consecutive days, which insured sterility. The last two methods are the most convenient and less apt to give contamination. The Nowak method of plating in the presence of a culture of *Bacillus subtilis* was only occasionally used. For the propagation of cultures ordinary slant or stab agar, with or without glycerin and glycerin bouillon, or serum glycerin bouillon are used. Holth and the English commission also recommended a liver bouillon culture as a suitable medium for the growth of the organism.

The writers have made extensive use of a gelatin agar shake, with or without bouillon, for the isolation of the organism. The media are composed of gelatin 40 parts and agar 60 parts, or gelatin 40 parts, bouillon 10 parts, and agar 50 parts. (Pl. XXVIII, fig. 2.)

Bouillon.—The inoculation of 3 per cent glycerin bouillon causes the liquid to become slightly cloudy after several days' growth and in many instances a very fine granular film is observed on the surface. Turbidity increases, but after several weeks the growth settles and the medium becomes clear. A sediment is noticed in a few days after inoculation, especially when sown heavily. This sediment as a rule becomes stringy, but in some cultures this is not so marked. Cultures in bouillon or serum bouillon often leave a bluish line or ring on the tube at the point where the surface of the culture comes in contact with the side of the tube. The reaction is neutral to litmus. Nowak considers bouillon a very suitable medium for the growth of this organism. The English workers claim that the addition of 1 per cent grape sugar makes the growth more luxuriant.

Slant agar (with or without glycerin).—In cultures sown from subsurface colonies or from tissues, evidences of growth are not observed for at least 7 days, usually 10 to 12 days, at which time a watery film appears which is soon transformed into a grayish glistening growth in direct light and light bluish-green in transmitted

PLATE XXVII.—BACILLUS ABORTUS FROM CATTLE.

FIG. 1.—Cover-glass preparation from an 8-days-old culture; stained with dilute carbol-fuchsin.

FIG. 2.—Cover-glass preparation from an 8-days-old culture; stained by Gram's method and counterstained with dilute carbol-fuchsin. On the same film are avian tubercle bacilli used as a control, which are seen to retain the Gram stain.

FIG. 3.—A 4-months-old culture showing involution forms; stained with dilute fuchsin.

These camera lucida drawings were reproduced at the level of the stand with Zeiss No. 4 compensating ocular and 2 mm. oil immersion objective.

PLATE XXVIII.—CULTURES OF BACILLUS ABORTUS.

FIG. 1.—An 8-days-old culture on 3 per cent glycerin-agar; originally obtained from the stomach contents of an aborted fetus.

FIG. 2.—A 10-days-old gelatin agar shake culture from the vaginal discharge following abortion.

FIG. 3.—A 3-weeks-old potato culture of *Bacillus abortus*, showing the similarity in growth to *B. mallei*.

PLATE XXIX.—COLONIES OF BACILLUS ABORTUS ON PLATE AGAR.

FIG. 1.—The two large colonies are surface growths of *Bacillus abortus;* the others are submerged colonies. All colonies in this figure are magnified 20 times.

FIG. 2.—Agar plate 10 days old, showing growths of *B. abortus* as small colonies. The large colonies and spreading growths are contaminating organisms.

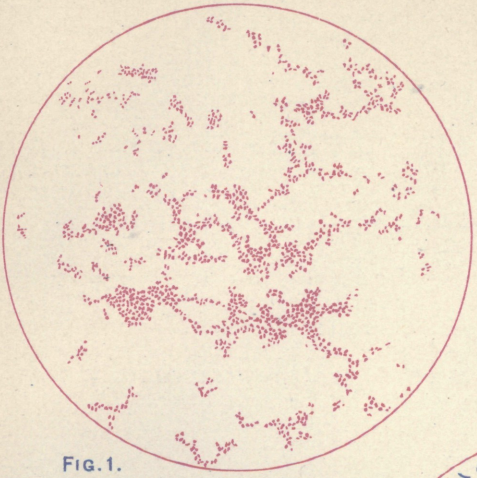


FIG. 1.

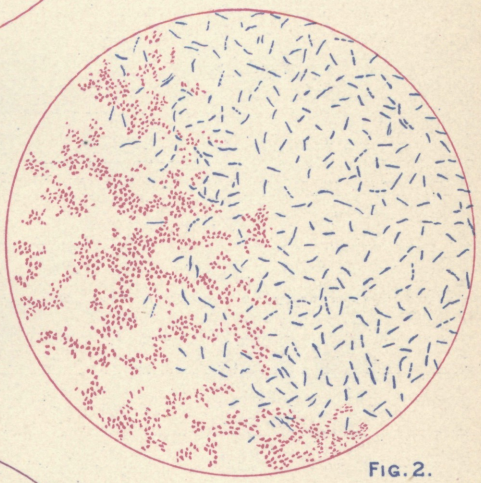


FIG. 2.

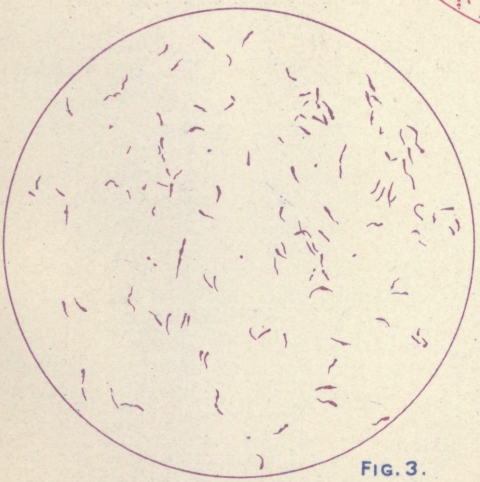


FIG. 3.

BACILLUS ABORTUS FROM CATTLE.

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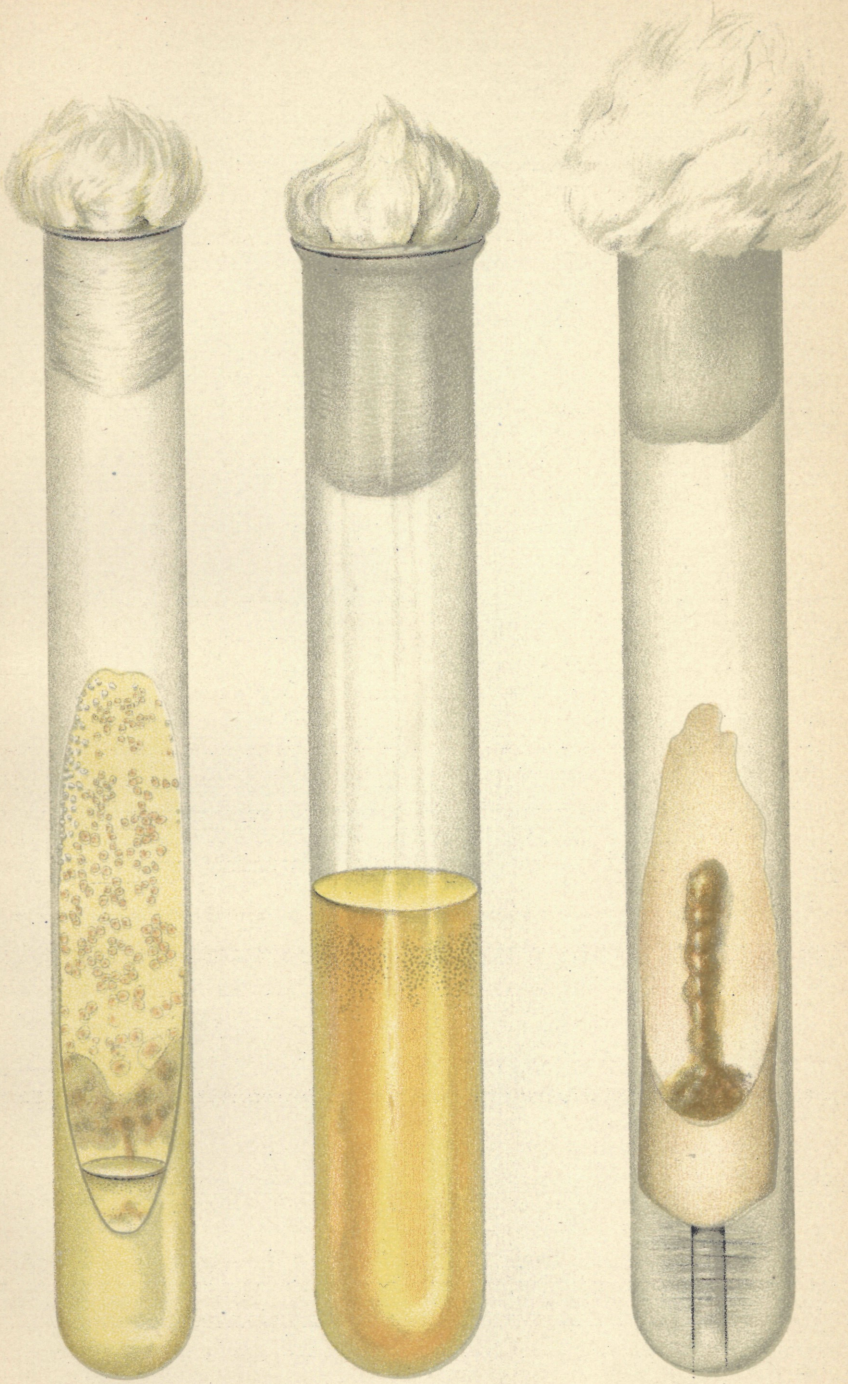

FIG. 1.

FIG. 2.

FIG. 3.

CULTURES OF BACILLUS ABORTUS.

Haines, del.

BREUKER & KESSLER CO. PHILA.

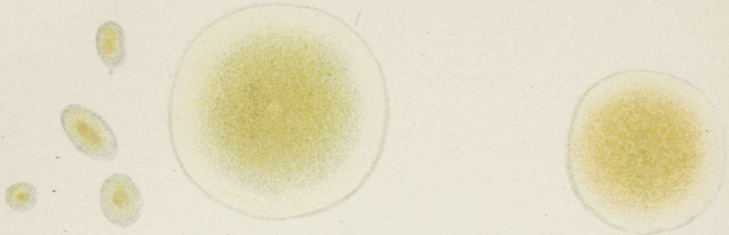

FIG. 1.

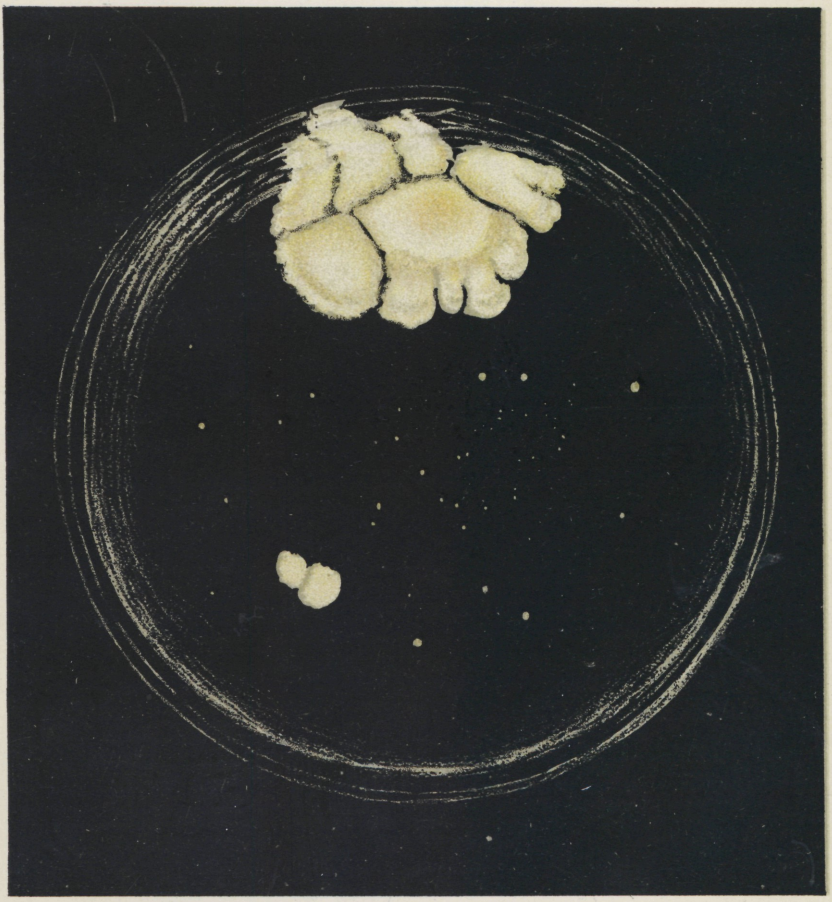

FIG. 2.

COLONIES OF BACILLUS ABORTUS ON PLATE AGAR.

(Large colonies and spreading growths in lower figure are contaminating organisms.)

Haines. del.

BREUKER & KESSLER CO. PHILA.

light. The cultures soon take on a yellowish-brown appearance, beginning at the center and becoming darker with age. In thinly seeded cultures there appear small glistening, round, raised colonies showing a grayish center and a bluish-green edge, which have very little tendency to spread. They vary in size from a pin point to 4½ millimeters. The individual colonies soon show a yellowish-brown center, the entire growth becoming darker in appearance, similar to that described above for the diffused colonies. (Pl. XXVIII, fig. 1.) The water of condensation shows a grayish sediment and a fine granular film on the surface which is easily disturbed. Growth on this medium is often very glutinous. Cultures made from aerobic growths and especially from slant agar show a luxuriant growth in 2 to 3 days. Individual colonies on agar plates present the same appearances as those described on slant agar. (Pl. XXIX.)

Stab agar.—Cultures on this medium show sacculatelike growths developing along the line of inoculation within 2 or 3 days. The growth on the surface, which is more profuse, appears at first glistening grayish white, later it becomes dirty gray, and finally brownish-yellow in color.

Gelatin at room temperature does not appear to be a very good medium for the growth of this organism. On several occasions, however, we succeeded in growing *Bacillus abortus* in this medium. The medium was not liquefied; growth was very slow.

Potato.—This culture medium has given variable results. It must be stated, however, that ofttimes the organism grows well on potato. It shows a glistening honeylike growth resembling somewhat the appearance of *Bacillus mallei.* (Pl. XXVII, fig. 3.) This similarity has been observed by most workers.

Milk.—While the organisms multiply in this medium, no perceptible change is noticed as a result of such multiplication. The reaction is neutral or slightly acid.

Fermentation tubes.—No gas is produced in dextrose, saccharose, or lactose. The reaction after several days is either neutral or alkaline to litmus. The growth is most prominent in the neck of the tube, the bulb later becoming cloudy; the closed arm, however, in all instances remained clear, even if organisms were shaken into it.

Tenacity.—Three-day-old agar slant cultures in test tubes washed with 0.85 per cent salt solution and heated to 55 to 56° C. in a water bath for three-fourths of an hour were not killed; exposure to the same temperature for one hour, however, resulted in the death of the bacilli.

Cultures grown like the above, but poured together in a liter flask and subjected to a temperature of 55 to 56° C., were not destroyed at the end of an hour, but were killed in 1½ hours. A bouillon culture heated to 70° C. for 7½ minutes failed to grow when reinocu-

lated. A temperature of 60° C. killed in 15 minutes cultures grown on slant agar and washed with sterile salt solution.

Bang found viable organisms in a uterine exudate kept in an ice chest for 7 months. Nowak reports that he obtained subcultures from a year-old culture.

Holth obtained a culture from material from fetuses kept at 2 to 4° C. for 8 months, and also from a series of pure cultures kept at room temperature for 9 months, but no growth could be obtained at the end of 1 year.

The writers have found this quality of the organism a variable one. Cultures on agar slants with paraffined plugs, kept at room temperature, have remained alive for 6 months. Cultures kept under the same condition for 9 months were no longer viable. The same observations were obtained with cultures stored in the ice chest for a similar length of time. Bouillon cultures kept at room temperature were still viable after 8 months.

BACTERIAL FLORA OF THE VAGINA.

Preliminary to our investigation of abortion a study was made of the bacterial flora of the bovine vagina in order to ascertain the character and frequency of the organisms present. The vaginas of nine animals were examined, six while alive and three after they had been slaughtered for beef, all the cows coming from a herd known to be free from infectious abortion. In all instances the search for *Bacillus abortus* gave negative results. Among the most frequently observed species which have been definitely determined are *Bacillus coli*, *Bacillus subtilis*, *Streptococcus pyogenes albus*, *Streptococcus pyogenes bovis*, *Pseudomonas pyocyanea*, *Bacillus mesentericus*, *Bacillus vulgaris*, *Cladothrix nonliquefaciens*, and *Sarcina subflava*.

The number of undetermined species is much larger and includes the following: A nonmotile, sporeless, liquefying bacterium occurring in chains of 6 or more cells, and frequently single, 2 to 3.5 μ long and 0.8 μ wide; a motile, spore-bearing, nonliquefying bacillus, 1.8 μ long and 0.6 μ wide; a Gram positive diplococcus about 1.2 μ in diameter, with slight ability to liquefy gelatin, but capable of producing dark red pigment on various culture media; a nonmotile bacterium varying in length from 2 to 6 μ and in width from 0.8 to 1.5 μ, with many involution forms; a Gram negative, nonchromogenic diplococcus about 1 μ in diameter, capable of liquefying gelatin and coagulating milk; a spore-bearing, liquefying bacillus, with rounded ends, occurring in pairs of short chains of 3 or 4 elements without the coagulation of milk; a nonmotile, nonspore-bearing, liquefying bacterium 2 to 3 μ long and 1 μ broad; an aerobic, motile, liquefying bacillus, with rounded ends, resembling in appearance and size the

Bacillus suisepticus; an aerobic, liquefying staphylococcus which failed to produce pigment on any culture medium; and several other organisms which have not as yet been studied.

While there was a certain uniformity in the species revealed, there were many types of organisms present that were probably transient and the result of contamination through the vulva. All of these different organisms were not present in every case, and in many instances the species mentioned was found only in individual cases.

INFECTIVENESS.

That the *Bacillus abortus* of Bang is capable of producing abortion experimentally in cattle, sheep, swine, goats, mares, guinea pigs, and rabbits by feeding and by intravenous injections has been satisfactorily proven by Bang, Nowak, McFadyean and Stockman, MacNeal and Kerr, Good, and others.[3]

Of the other pathological changes produced by this organism, MacNeal and Kerr mention that in one of their guinea pigs inoculated subcutaneously, there appeared at the point of inoculation a purulent inflammation. Holth was able to kill white mice and spotted rats in two and three days, respectively, by intraperitoneal inoculation of ¼ c. c. of a serum bouillon culture, and found the organism in the blood. Such was not our experience until quite recently, when a very virulent strain of the organism was isolated which killed mice in 48 hours. With all other strains white mice inoculated intraperitoneally failed to die. We were, however, successful in producing an enlarged nodular spleen in these animals at the end of 2½ to 3 months. Spotted rats were not used, but white rats failed to show any ill effects at the end of 2 months from such inoculation.

In two pregnant guinea pigs inoculated subcutaneously by the writers with 1 and 2 c. c., respectively, of a 7-day-old bouillon culture, both of which aborted 10 days after injection, a considerable tumefaction appeared at the point of inoculation. This is not an infrequent occurrence in guinea pigs inoculated subcutaneously. One of these guinea pigs when chloroformed 2 months after inoculation still showed a small, bean-size, soft, caseated mass, with the surrounding tissue of a brownish-yellow color. From this caseated mass pure cultures of the *Bacillus abortus* were obtained direct on slant agar, and in shake cultures there appeared at the same time subsurface and surface growths. The uterus of this guinea pig was inflamed and contained a brownish-yellow viscid material from which *B. abortus* alone was isolated.

Smith and Fabyan,[28] working with necrotic cotyledons from an aborting cow, and suspecting the possibility of tuberculosis, inoculated a portion of their material into a guinea pig. Several months

after the inoculation they chloroformed the animal, and to their surprise found an engorged spleen, enlargements of subcutaneous and visceral lymph nodes, and infiltration and yellowish nodules of the liver. Inoculation with organs from this animal and inoculations with cultures of *Bacillus abortus* from several sources gave these authors similar lesions, with the addition that some guinea pigs so inoculated showed lesions in the lungs, testes, ribs, and eyes.

Various workers in the laboratories of the Bureau of Animal Industry, including Theobald Smith, have for many years observed that guinea pigs inoculated with milk for the purpose of detecting or isolating the tubercle bacillus, and autopsied at the end of 2½ or more months, showed lesions resembling to some extent those of tuberculosis, but in which no tubercle bacilli could be demonstrated. It remained, however, for Schroeder and Cotton,[22] of this bureau, to isolate and describe the organism that was responsible for these lesions. At the same time the present writers were working on various phases of contagious abortion in cattle, and had just succeeded in growing *B. abortus* on slant agar. The similarity of the Schroeder and Cotton organism, known to them as bacillus 637, and the *B. abortus* was so marked in both morphology and cultural characteristics as to lead the writers to suspect that these two organisms were identical, and this suspicion was strengthened by the fact that Schroeder and Cotton had obtained a good portion of their milk from which they isolated their organism by guinea pig inoculations from the same institution from which the writers were receiving a great deal of the material for their work on contagious abortion. The writers at once inoculated guinea pigs with pure cultures of *B. abortus*, with the result that similar lesions were produced. The reaction to the Gram method of staining still kept these two organisms apart, since Schroeder and Cotton described their bacillus 637 as Gram positive, while *B. abortus* has given the writers and other workers negative results by this method. Cultures were then exchanged, with the result that both bacillus 637 and *B. abortus* failed to take the stain with the Gram method. The organism isolated from milk was compared with the *B. abortus* from several laboratories, and no difference could be detected in either cultural characteristics or morphology; and finally the use of bacillus 637 as an antigen in applying the complement-fixation test to sera from naturally and experimentally infected animals with *B. abortus* established the identity of these organisms beyond a doubt.[16] The fact that the organism can produce characteristic lesions in guinea pigs makes the isolation of the *B. abortus* even from contaminated material a relatively simple matter.

Probably the most important and comprehensive facts which have been demonstrated in connection with this disease are the discovery

that the abortion bacillus is eliminated with the milk of infected cows, and, secondly, that this bacillus is found in the tonsils of children, presumably as the result of drinking such infected milk. The frequency of the presence of *B. abortus* in a food product like milk and the ability of the organisms to produce lesions in guinea pigs, pregnant cows, and other animals led at once to the thought that *B. abortus* might prove pathogenic for human beings. As a result our endeavors were directed along three lines: First, to obtain sera promiscuously from human beings, and in case of positive reactions to learn more about the person whose serum showed the reaction; second, to obtain samples of milk from women in order to examine it for this bacillus; and, third, to obtain tonsils from milk-consuming children at the various children's hospitals and inoculate such material into guinea pigs. Material for these lines of work was not forthcoming as fast as desired. Out of 42 sera from human beings no positive results were obtained by either the complement-fixation or agglutination tests, although in similar tests made by Larson 3 out of 100 specimens of sera gave positive results. No samples of human milk have thus far been obtained. Out of 56 tonsils and adenoids inoculated into guinea pigs, tonsil No. 3 produced nodular areas in the liver, but cultures from this organ remain sterile. Tonsils from case No. 8 inoculated into 2 guinea pigs showed in one of them after 3 months distinct lesions of infection in the liver, spleen, and testicles, and *B. abortus* was obtained from the lesions.

That the virulence of the different strains varies has been our constant experience; in fact, within two or three generations the same strain seems to vary considerably. Of 4 pregnant rabbits inoculated with a strain of *B. abortus*, 1 intravenously and 1 subcutaneously injected rabbit aborted in 13 and 17 days, respectively, after the inoculations. One rabbit that received intravenous inoculation was apparently not pregnant or else aborted and did away with the fetuses. The fourth gave birth to a litter of young ones 30 days after inoculation. The same experiment was repeated with the same strain after two generations of subsequent growth on artificial media, but with negative results. The difference noted above between Holth's white-mice inoculations and our earlier experience with white mice may also be explained by the difference in virulence and probably also the difference of resistance of the inoculated animals. Dogs, cats, and chickens fed and inoculated with this organism failed to show any evidence of infection.

That the *Bacillus abortus* might possibly play a part in the production of disease of newly born calves, especially white scours and septicemia, is suggested by the fact that these diseases are often found coexisting with infectious abortion in a herd. Jensen [11] found 1 out of 208 cases of white scours directly traceable to the abortion

bacillus. Holth was unable to obtain either a complement-fixation or agglutination reaction from calves that died of diarrhea within the first two weeks of life. The writers obtained positive complement-fixation (0.01 c. c.) and agglutination (0.002 c. c.) reactions from a prematurely born calf in an infected herd which died of scours when 10 days old. Experimentally, however, we were unable to produce any noticeable ill effects on two 3-day-old calves that were fed each with 90 c. c. of a *B. abortus* bouillon culture in milk for a period of 3 days.

SUSCEPTIBILITY.

Cows of all ages are more or less susceptible to the disease. (See Tables 1 and 6.) Animals in their first or second pregnancies are more apt to abort if exposed than at any other time. Cows that have aborted once may abort a second time; abortion in the same cow more than twice is an unusual occurrence. Heifers from aborting mothers may be less susceptible than those born to noninfected dams; in fact, in one herd under observation such animals have shown a pronounced resistance to the infection. Cows and heifers reared in a noninfected environment very often abort shortly after being brought to infected premises.

AGENTS OF DISSEMINATION.

Even before the discovery of the specific organism various observers had recognized the infective character of the afterbirth, fetus, and vaginal discharges from the aborting animals. Franck and Bräuer were able to produce abortion experimentally with afterbirth and vaginal discharge from aborting animals. The fact that the specific agent has now been isolated in a majority of cases from the gastro-intestinal tract, and at times from the liver and general circulation of the aborted fetus, places the product of conception prominently in this list. Milk has recently been added to the number of agencies by which the virus is eliminated from the body, as has already been referred to above. Thus the stall, litter, bedding, feed, water, pastures, stockyards, transportation cars and boats, attendants, their clothing, and various other objects can very readily become contaminated with one or more of the above-mentioned sources of infection, thereby acting as disseminators of the disease to other animals. The manure, if not composted properly and the outermost layer disinfected, may spread the disease to distant points. McFadyean and Stockman mention the possibility of foxes aborting as a result of infection with *Bacillus abortus* and acting as carriers of the infection. The same holds true in case of other animals, and especially with sheep and goats, since these animals have been claimed to abort not only as a result of experimental inoculation but also by pasturing or feeding on infected premises.

NATURAL MODE OF INFECTION.

This important phase of the disease is and has been responsible for a great deal of discussion and diversified opinions.

Artificially the disease can be produced by introducing the virus into the body by way of the digestive tract and the vagina and by intravenous and subcutaneous inoculations. With the first two methods we are principally concerned, since the intravenous inoculation, while giving the greatest number of positive results, is purely an experimental method. The latter statement holds true for the subcutaneous inoculation, which gives few positive results, and, as proven by the English commission, requires rather too large a dose in the cow to be a probable occurrence naturally.

Nocard has also suggested the respiratory tract as a probable path of infection. The infection per orem has been considered an important natural avenue of infection by Bang,[2] Zwick,[29] Holth, and others, and particularly so by McFadyean and Stockman. W. L. Williams,[25] one of the stanchest supporters of the infection per vagina, claims that Bang and other workers, in their experiments to prove infection through the mouth, do not show how the infection of the vagina was excluded, stating that these ruminating animals upon which the experiments were conducted could easily transfer the infection from the oral cavity to the vagina by licking, a habit to which many cows are addicted. This same argument is, however, equally applicable in favor of infection through the mouth; that is, an animal infected artificially or naturally through the vagina may lick that organ or discharges from the same and thereby introduce the virus into the oral cavity. In favor of the infection by means of the vulvo-vaginal tract there is a great deal of clinical evidence, most conspicuous among which is the oft-quoted and classical case of Paulsen, where on a farm that had always been free from the disease 7 out of a herd of 16 cows were served by a bull from infected premises, causing abortion in 5 cows within 10 weeks, in one 3 months, and in one 4½ months after copulation.

Williams[26] bases his treatment of abortion, which is given in the publication cited, on the ground that infection takes place through the vaginal tract. Holth, quoting Wall's histologic findings, states that the latter found the mucous membrane in the spaces between the cotyledons to be affected in three cases, thereby indicating that the infection occurred through the cervix, since infection through the blood would locate itself first in the cotyledons.

Another class of abortion where the bull is most probably the carrier of the infection, and principally during copulation, is that occurring among range cattle. But while the bull during copulation can very easily transplant infection into the cervix and uterus, it

is hardly probable that infection from the vagina would readily pass through the rigid plug in the cervix after conception. If infection does take place after conception it probably does so by means of the lymphatic system of the vagina.

On a farm where the disease is not known to exist a new cow may be brought into the herd, or one of the cows of the herd may be taken to a bull on infected premises for service, and it often happens that not only the newly introduced cow or the one bred to the bull but others in the herd will drop their calves prematurely in quick succession, or give birth to calves that die at the age of several days. In such cases the infection must have taken place after conception, either by the genitals coming in contact with stalls, bedding, etc., contaminated with virus, or else the bacilli were introduced through the digestive tract by feed or drinking water containing the specific organism. And from the vast number of experimental abortions produced by introduction of virus through the mouth, particularly in animals such as rabbits, where the virus is introduced by tube and infection by vagina can be easily excluded, this means of infection must be given an important place, especially so until satisfactory experimental evidence is brought forward to the contrary.

PATHOGENESIS.

No matter what the mode of natural infection may be, it is obvious that the bacillus of abortion shows a predilection for the mucous membrane of the uterus. If the infection is introduced during copulation the virus can readily be deposited in the cervical canal by the penis of the bull, from where it can easily gain entrance to the uterus. If the infection, on the other hand, finds its way into the alimentary tract, or into the genital tract after the closing of the os uterus, it is necessary for the virus, in order to reach the uterine mucous membrane, to be taken up by the lymphatics, whence it finds its way into the circulation and is finally planted on the mucous surface of the uterus. The multiplication and activities of this organism result in the injection and catarrh of the uterine mucous membrane and the formation of the characteristic yellowish to dark brown tenacious exudate on the surface, and in some instances an infiltration of the submucosa. The intervention of the exudate, or the necrotic changes of the cotyledons sometimes found, interfere with the osmotic changes between the fetal and maternal membranes, thus producing the death and expulsion of the fetus. The death of the fetus may also be occasioned by the entrance of the organism into the fetal circulation through the destroyed placental capillaries. The accumulation of a large amount of the exudate at the internal os may act as an irritant or as a solvent and cause the opening of the cervix and expulsion of

the fetus. Death of the fetus does not always cause its discharge, but it may be retained within the uterus, which results in its maceration or mummification.

The failure of aborting cows to breed may be explained by the persistence of the infection in the uterus, which prevents conception. However, failure to conceive can not always be traced back to infection with *Bacillus abortus*, as will be referred to later.

PATHOLOGIC ANATOMY.

The one constant and characteristic pathologic change present in practically all cases of infectious abortion is a yellowish to dark brown exudate found between the uterine mucous membrane and the chorion, varying in consistency from a mucopurulent to a tenacious gluey substance. McFadyean and Stockman suggested that the color is probably due to the chromogenic properties of the organism. The exudate contains grayish-white flakes and is composed of detritus, leucocytes, and epithelial cells in various stages of retrogressive changes from granular degeneration to necrosis. Bacilli of abortion are found lying free and within the leucocytes. The quantity of the exudate varies greatly from a small, almost unobservable amount, principally around the cotyledons in the vicinity of the internal os, to a large quantity covering the entire gravid uterus. In many cases the nongravid horn is similarly involved. McFadyean and Stockman record a twin pregnancy in which one of the horns showed considerable exudate, while the other was apparently normal. The nature of the exudate only becomes changed in cases of mixed infections, but this is an exceedingly rare occurrence, and when present is occasioned by other diseased conditions or by the failure to expel the fetus soon after the cervical canal is opened.

The uterine mucous membrane, while apparently normal in a number of cases, is frequently swollen, injected, hemorrhagic, or roughened by a serofibrinous exudate, and in some instances may even show necrotic areas. The outer walls of the uterus have never been found to be involved. The submucosa in some cases is edematous. The cotyledons seem to receive the brunt of the attack, being in a great majority of cases reddened, or on the other hand pale, soft, and necrotic, and nearly always surrounded by the abortion exudate. The chorion and chorionic tufts, besides showing a subchorial edema, may show lesions similar to those described for the uterus and cotyledons. Oftttimes, however, the chorioallantoic tissues may have a lusterless, dead, opaque shrunken appearance. McFadyean and Stockman have found one natural and one experimental case in which the chorion was thickened and leathery in appearance. The umbilical cord in a good many instances shows a serohemorrhagic infiltration, but may on the other hand be apparently normal. The fetuses show a sero-

sanguineous infiltration of the abdominal or thoracic wall, or both, but principally of the former. A sanguineous-gelatinous exudate may be observed in the peritoneal and thoracic cavities, which is more marked in the former.

Unless there are general manifestations of septicemia, macroscopic changes are as a rule not observed in the visceral organs, excepting the gastrointestinal tract. A catarrhal gastroenteritis is the usual alteration. The contents of this tract are yellowish to brownish orange, tenacious in consistency, sticking closely to the walls. Upon standing overnight there appears a supernatant amber-colored fluid with a viscid brownish-orange sediment. The stomach contents resemble the abortion exudate found in the uterus, but are at times more highly colored. Cultures made from such material will invariably bring forth colonies of *Bacillus abortus*. In 15 attempts we have never obtained the specific organism from the blood of a fetus. The liver furnished cultures in one instance and the stomach contents in 11. The fetus in rare cases may be macerated or may become mummified. The process of mummification does not take long. McFadyean and Stockman found a mummified fetus in an experimental case 138 days after the infection. The allantoic and amniotic fluids may be turbid and contain flocculi.

SYMPTOMS.

The period of incubation is another phase of the disease which varies within wide limits. The only definite information on this subject is found in the experimentally infected cases, unless we take the untenable ground that all infections are transmitted during copulation. From the artificially inoculated cases recorded by Lehnert, Bräuer, Bang, McFadyean and Stockman, and others we learn that the period varies from 1 to 33 weeks; McFadyean and Stockman's cases averaged 126 days. It matters little how the infection was introduced or whether the virus used was material from aborting cows or a pure culture, the period of incubation is irregular. The variation in part may be explained by the difference in virulence of the organisms and in the susceptibility of the individual. One must bear in mind, however, that the disease as a rule is chronic, and in such cases the disease processes have no doubt been existing long before abortion takes place.

Premonitory symptoms are not always observed, but when present are manifested two or three days before the expulsion of the fetus by swelling of udder ("making bag"), edematous swelling of the vulvar lips, reddening of and small inflamed nodular formations on the vulvo-vaginal mucosa, and the appearance of a mucoid or mucopurulent odorless discharge from the vagina. While all or most of these manifestations are present in a very large majority of cases of

infectious abortion they are not prima facie evidence that abortion will occur. The swelling of the udder would be noticed in non-milking cows only, and in heifers there is invariably a swelling of the bag beginning some months before parturition. The other symptoms are not infrequently observed in nonaborting animals. In a herd of 45 Holsteins, where the writers were conducting some experimental work, and where abortion and granular vaginitis were coexistent, several cows showed a discharge with reddening and nodular formation on the vulvo-vaginal mucous membrane. One case in particular showed a profuse vaginal discharge, intense inflammation of the vaginal mucous membrane, and swollen vulvar lips. The manager, an experienced dairyman, was warned of the approaching abortion, and he agreed that the fetus would be expelled within a day or two, but neither this cow nor other suspicious ones have as yet aborted. In an infected environment a large percentage of animals abort during the first or second pregnancy. Cows most often abort between the fifth and seventh months of gestation. Animals aborting for the first time usually do so at an earlier stage than do those with a history of previous abortion. In cases where the infection has persisted in the uterus, however, abortion occurs at an early stage of gestation. The abortion at a later stage in a subsequent pregnancy may be caused by a reinfection. Cows may abort a second time, but a third abortion in the same cow is rare.

There is no doubt that abortion during the early months occurs more frequently than is reported but is unnoticed and considered simply as "failing to catch." Should the abortion occur in the early months of gestation when the chorionic tufts are not yet fully developed, the fetus and its membranes are expelled at the same time and the act is attended by no systemic disturbance. However, if the abortion should occur in the late months of pregnancy, the afterbirth is generally retained, or at least not voided together with the fetus, and the abortion is attended with restlessness and pain on the part of the aborting animal.

The fetus is, as a rule, born dead, or if alive is usually weak and puny and dies within a few days with diarrheal symptoms or remains a runt. Nocard and others have reported that calves born alive before the full period of gestation has expired utter peculiar cries which simulate the howling of a rabid dog.

Following the abortion there is a dirty yellowish-gray, mucopurulent discharge which persists for two or more weeks. The retained placenta if not removed within a few days after the abortion may give rise to necrosis and subsequently to sapremia, or the changes may extend to the uterine mucosa, causing endometritis, and in some instances may involve the whole thickness of the uterus and even penetrate into the abdominal cavity and produce peritonitis. Chronic

metritis and pyometra may follow improper handling of the retained placenta. In a good number of cases cows may, to all appearances, recover from the effects of the abortion, the discharge and all inflammatory changes having ceased, but nevertheless they may fail to conceive and be brought to the bull several times before becoming impregnated, or may never again be successfully bred. Such condition is no doubt the result of retained placenta or endometritis in a great majority of cases. The failure to conceive for all time, or failure to conceive after one or more services, does not always mean that this condition is caused by an infection with *Bacillus abortus;* at least such inferences can not be drawn from our observations recorded in Table 1.

TABLE 1.—*Records of certain cows in two herds of 108 animals, in which infectious abortion has been prevalent for several years.*

Cow No.	Complement-fixation test.			Agglutination test.				History
	0.05	0.02	0.01	0.01	0.005	0.003	0.002	
1....	−	−	−	−	−	−	−	Calved about 1½ years before blood drawn for this test and has not conceived since.
2....	+	+	+	+	+	+	+	Dropped an 8-months fetus 10 months ago and has not conceived since.
3....	−	−	−	−	−	−	−	Calved 9 months ago; repeatedly bred since, but does not conceive.
4....	−	−	−	−	−	−	−	Do.
5....	−	−	−	−	−	−	−	Calved 7 months ago; repeatedly bred since but does not conceive.
6....	−	−	−	−	−	−	−	Calved 1 year ago; repeatedly bred since, but does not conceive.
7....	−	−	−	+	−	−	−	Do.
8....	−	−	−	−	−	−	−	Aborted a 2-months fetus 6 months ago; bred, but does not conceive.
9....	+	+	+	+	+	+	+	Calved 13 months ago; bred repeatedly, but does not conceive.
10....	−	−	−	−	−	−	−	Has not conceived for 16 months, even though bred repeatedly.
11....	+	+	+	+	+	+	+	Do.
12....	?	−	−	−	−	−	−	Do.
13....	−	−	−	−	−	−	−	Has not conceived for 8 months, even though bred repeatedly.
14....	−	−	−	+	−	−	−	Never aborted; has given birth to 11 calves. In calf now, but was bred three times before conceiving.
15....	−	−	−	−	−	−	−	Never aborted; has given birth to 3 calves. In calf now, but was bred 3 times before conceiving.
16....	(1)	(1)	(1)	+	+	+	+	Aborted last 2 calves; first at 5 months, second at 7 months. In calf now, but was bred twice before conceiving.
17....	(1)	(1)	(1)	+	+	+	−	Never aborted; has given birth to 10 calves. In calf now, but was bred 6 times before conceiving.
18....	+	−	−	+	−	−	−	Aborted first calf; has given birth to 11 since. In calf now, but was bred 4 times before conceiving.
19....	+	+	−	+	+	+	+	Never aborted; has given birth to 6 calves. In calf now, but was bred 4 times before conceiving.

[1] No test.

TABLE 1.—*Records of certain cows in two herds of 108 animals, etc.*—Continued.

Cow No.	Complement-fixation test.			Agglutination test.				History.
	0.05	0.02	0.01	0.01	0.005	0.003	0.002	
20....	(1)	(1)	(1)	−	−	−	−	Aborted first calf at 5 months; has given birth to 6 calves since. In calf now, but was bred twice before conceiving.
21....	+	−	−	+	?	−	−	Never aborted; has given birth to 9 calves. In calf now, but was bred twice before conceiving.
22....	−	−	−	−	−	−	−	Never aborted; has given birth to 4 calves. In calf now, but was bred 3 times before conceiving.
23....	−	−	−	−	−	−	−	Never aborted; has given birth to 1 calf. Has been bred 3 times and is not yet with calf.
24....	+	+	+	+	+	+	+	Never aborted; has given birth to 1 calf. Was bred 3 times before conceiving.
25....	−	−	−	−	−	−	−	Never aborted; has given birth to 9 calves. Was bred 3 times before conceiving.
26....	+	+	+	+	+	+	+	Aborted her first calf; has given birth to 2 calves since. Bred twice before conceiving.
27....	−	−	−	−	−	−	−	Never aborted; has given birth to 6 calves. In calf now, but bred 3 times before conceiving.
28....	.....	+	.....	+	+	+	+	Had 3 calves; aborted her fourth at 3 months, 4 months ago, and previous to her last conception she was bred 4 times.

[1] No test.

For interpretation of tests given in this table, see page 173.

This table shows that a number of cows that failed to conceive after one or more services did not give any reaction to the agglutination and complement-fixation tests. Hence the organism could not have been doing much damage to such a large percentage of animals so affected without their bodies defending themselves by producing bacteriolysins or agglutinins or both. The cause of such sterility must therefore, in some animals at least, be looked for elsewhere.

DIAGNOSIS.

The premonitory symptoms as enumerated above are not always followed by abortion, and even when they are so followed they are not indicative of the presence of infectious abortion. Granular vaginitis, which has been accused by many workers as being responsible for abortion, may exhibit similar manifestations, but in these cases when abortion does occur the general opinion among most authors in recent literature is that such abortion is not directly traceable to the granular vaginitis, but to infection with *Bacillus abortus*. When a herd has a history showing that a number of cows abort during a season, one is justified in concluding for all practical purposes that he is dealing with infectious abortion. Such a diagnosis is also indicated if the disease was not known to exist on the farm until the introduction of a new bull or even a new cow, after which several cases of abortion occur.

W. L. Williams points out that abortions due to mechanical injuries are so rare that they may safely be eliminated as causative factors in most cases. He examined gravid uteri of 1,736 pregnant cows and heifers, and in spite of being on the lookout for injuries to the genital organs, he could not find a single case. He emphasizes the fact that these animals had been driven to railroad stations, loaded into cars, transported for many miles, and in many instances unloaded into stockyards, jostled and driven, and finally slaughtered. If such treatment does not induce abortion, the ordinary accidents, such as slip or jam or prod by attendant or riding by another cow, should not be held responsible for the slinking of the calf in many cases.

The constant presence of the abortion exudate may be of aid in making a diagnosis, but here we may be hampered by the chance of external contamination if the examination is not made soon after abortion. Where infectious abortion can not be diagnosed from history and clinical manifestations the following methods of diagnosis have been recommended: (1) Bacteriologic; (2) serologic; (3) allergic.

BACTERIOLOGIC DIAGNOSIS.

Microscopically, most satisfactory results are obtained by examining smears made from the gastrointestinal contents of the fetus or from the discharge of the vagina before and soon after abortion. The presence of small, Gram negative, almost coccuslike bacilli in clumps is strongly suggestive of *Bacillus abortus*. Cultures made from similar material give in the majority of positive cases cultures of *B. abortus*, but negative results must not be taken as final proof of the absence of infectious abortion. Inoculation of guinea pigs either subcutaneously or intraperitoneally will in many instances produce tuberculouslike lesions in three or more months, from which *B. abortus* may be recovered. The last two methods are not practicable because of the time which elapses before results are obtained, this being especially true of the animal inoculations.

SERUM DIAGNOSIS.

Following the success of serum diagnosis in other diseases, especially in glanders, various workers in veterinary pathology have applied the serologic tests—agglutination and complement-fixation—to abortion. Among these are McFadyean and Stockman, Grinsted, Holth, Wall, Brüll, and Zwick. In this country Larson was the first to report results of the complement-fixation test.[a] Both of these tests have been used in this and in other laboratories of the country. The serum is obtained, as in the case of glanders in horses, by bleeding

[a] Since this was written bulletins on the diagnosis of infectious abortion have been issued by Hadley and Beach, of the Wisconsin Experiment Station, and Surface, of the Kentucky Experiment Station.

the suspected animal from the jugular vein, allowing the blood to stand until the clear serum separates from the clot. Such serum may then be immediately used or placed in an ice chest, where it will remain unaffected for several months. One part of a 5 per cent solution of carbolic acid added to 9 parts of serum will preserve the serum, but ordinarily this is not necessary, and in fact uncarbolized clear serum is preferred by the writers for the application of the tests.

THE AGGLUTINATION TEST.

Various limits have been placed on the lowest dilution of serum from animals which should be considered not as normal agglutinins, but as the result of infection with *Bacillus abortus*. MacFadyean and Stockman have placed the dilution at the very low limit of 1:25, but they conclude that the agglutination test can not be regarded as free from great risk of error. Grinsted considers that normal sera never agglutinate in dilutions higher than 1:30. Brüll concludes that sera from normal cattle agglutinate *B. abortus* in dilutions not higher than 1:32. Sera from animals affected with *B. abortus* show with few exceptions titres of 1:120 to 1:16,000. He believes that a titre of 1:64 can give no definite conclusions, since he obtained such titre in one case of abortion and in two normal animals.

Zwick states that just prior or for a time after abortion the serum will show agglutinating value in dilutions of 1:100 to 1:10,000, and that animals known to come from abortion-free environment never agglutinate in dilution of 1:100.

Both Holth and Wall suggest the advisability of using both the complement-fixation and agglutination tests, since the presence of normal agglutins are not apt to be coincidently present with normal bacteriolytic amboceptors. Wall gives the following formulas for reactions:

Positive reaction { Agglutination $\gtrless 0.05$ [a] ; Complement-fixation $\gtrless 0.05$

Negative reaction { Agglutination $= 0$; Complement-fixation $= 0$

or { Agglutination $\gtrless 0.05$; Complement-fixation $= 0$

or { Agglutination $= 0$; Complement-fixation $\gtrless 0.05$

The technic used by all the above-mentioned workers consists principally in making a series of dilutions in agglutination fluid and allowing them to stand for various lengths of time in the incubator and

[a] $\gtrless 0.05$ represents a serum whose titre is equal to or greater than 0.05.

then reading the results. In our work we have followed the same technic as outlined by Muller, Miessner, and Pfeiler and also described by Mohler and Eichhorn[18] for glanders, differing from them principally in not reading the agglutinating titre of a particular serum according to dilution but considering the quantity of serum used as an index of the agglutination value. This was first suggested by Wall. For example, a serum is diluted to one-tenth of the original strength; then if 0.02 c. c. of this basic dilution will agglutinate in a tube to which 1 c. c. of agglutinating fluid is added, the agglutinating titre of such serum would be considered 0.002 instead of 1:500, because, should 1 or 2 c. c. or even 4 c. c. of salt solution be added to the tube containing 0.02 c. c. of the one-tenth dilution, there will still be a complete agglutination, whereas the dilution would now be 1:1,000 or 1:1,500 or higher. Even the addition of 2 c. c. of the agglutinating fluid instead of 1 c. c., which would make the dilution 1:1,000, does not affect the agglutination power of the serum (0.02 c. c. of a one-tenth dilution).

The agglutinating fluid is made from a week to 10-day old slant agar cultures (other workers use washed *Bacillus abortus* grown in fluid medium [serum bouillon]), washed with carbolized salt solution. Killing the culture by heating has not given us as good results as the fluids preserved simply by carbolization (0.5 per cent). More than one strain of the *B. abortus* has been used for our work, the best results being obtained by using three or four strains. The opacity of the fluid thus obtained is compared with the old titred agglutinating fluid. This is accomplished by filling two beakers of equal dimensions to the height of 2.5 to 3 centimeters with the old and new fluids, respectively. The beakers are placed on clear-cut printed matter, and by looking through them from above the density of the fluids can very readily be compared. Should the printing under the new fluid appear clearer than the old titred fluid, some more heavy bacterial suspension or bacterial growth should be added until the new fluid has the same opacity as the old. Should the new fluid be too turbid, it is thinned down by the addition of carbolized salt solution. The fluid now of the proper opacity is then subjected to titration by testing two or more sera, preferably of known agglutination titre with both new and old fluids, and if the results are similar the new fluid may be considered as suitable for the application of the agglutination test for infectious abortion. The fluid keeps well for at least two months when kept at a temperature of 4 to 6° C.

In making the test a series of six round-bottom test tubes that can be used for centrifugalization are placed in a test-tube stand, with conical-shaped holes at the bottom. One part of the serum to be tested is then added to 9 parts of carbolized salt solution. This known basic dilution is drawn into a 1 c. c. pipette, graduated into

hundredths, and the following amounts are placed in each of the six tubes:

First tube, 0.15 c. c., representing 0.015 of the original serum.
Second tube, 0.1 c. c., representing 0.01 of the original serum.
Third tube, 0.05 c. c., representing 0.005 of the original serum.
Fourth tube, 0.03 c. c., representing 0.003 of the original serum.
Fifth tube, 0.02 c. c., representing 0.002 of the original serum.
Sixth tube, 0.01 c. c., representing 0.001 of the original serum.

To each of these tubes 1 c. c. of the agglutinating fluid is added. Tubes are thoroughly shaken to wash off the serum that may be clinging to the sides of the tubes, and then they are placed in the incubator for from 20 to 30 minutes. This is followed by centrifugalization for 10 minutes at a speed of 1,600 revolutions per minute. The tubes are then placed back into the test-tube rack and held toward the light so that the bottom of the tubes can be read. Agglutination is indicated by the appearance of a more or less regular, thinly spread film at the bottom of the tube. The exact manner in which the agglutinated bacteria are distributed over the bottom of the tube depends largely upon the precise shape of the bottom. When the bottoms of tubes show a compact, round, grayish-white sediment of bacteria, it indicates failure of the serum to agglutinate. While results can be read soon after removal from the centrifuge, it is advisable to examine the tubes again after they have been allowed to stand overnight at room or ice-chest temperature, although in over 400 cases tested in this manner no difference was noticed between the results read soon after the centrifugalization and those read the day following.

While the serum of no known normal cow or steer has thus far given us a complete agglutinating value of 0.01 or complete agglutination in the second tube of the above series, still it is not advisable to consider any cases with such titre as positive on the strength of the agglutination test alone. At present to call a case positive we demand that the serum should show a titre of at least 0.005. Especially is this procedure advisable in case a herd of cows are tested and not one serum of the lot gives a titre higher than 0.01. Agglutinins may exist before abortion in serum of animals and especially so soon after abortion. They may persist for two years, but they gradually begin to decrease several months after the abortion. Grinsted concludes that serum showing a titre of 1:1,800 indicates a recent infection, and therefore a probable case of abortion if the animal is pregnant.

THE COMPLEMENT-FIXATION TEST.

McFadyean and Stockman were the first to use the complement-fixation test as an aid in diagnosing infectious abortion, but Wall and Holth did exhaustive work with and perfected the technic of

the complement-fixation test in this disease. In this country Larson[18] used practically the same method as described by both Wall and Holth with but slight modification, and the procedure in this laboratory[17] differs only in detail from that first described by Wall.[a]

The hemolytic system.—Since this laboratory is applying almost daily the complement-fixation test for glanders, it was deemed advisable to use the same hemolytic system for the test in infectious abortion as in glanders; therefore, washed red cells of sheep, hemolysins furnished by sera of rabbits immunized against sheep erythocytes, and complement from guinea pigs constitute the active ingredients of the hemolytic system.

In our work on this disease (Tables 3, 4, and 5) sensitized red blood cells are used. Sensitizing the red cells is accomplished by adding to washed red cells of sheep sufficient hemolysin to sensitize the desired quantity of blood. Thus, to 5 c. c. of washed red blood cells, which make 100 c. c. of 5 per cent solution (the desired suspension for the work in glanders, as indicated in Bulletin 136), there would be required 0.05 c. c. of a hemolysin which is used in the glanders test in dilutions of 1:2,000. The 0.05 c. c. of such hemolysin (rabbit serum) is dissolved in a suitable quantity of normal salt solution (about 50 c. c.); to this the 5 c. c. of washed sheep red cells are added, and the product is incubated at 37.5° C. for 1 to 1½ hours. The whole product is then centrifugalized, the supernatant fluid drawn off, and the red cells washed by adding salt solution and again centrifugalizing; this is again repeated. After the supernatant fluid is drawn off for the third time the tube with the washed red cells at the bottom is placed in the ice chest and dilutions made when desired. When the red cells are not sensitized the following table from Holth represents a scheme for titrating the hemolysins:

TABLE 2.—*Method of titrating hemolysins.*

Tube.	Complement, 1:4 dilution.	Hemolysin, diluted 1:100.	Salt solution, 0.9 per cent.	Sheep corpuscles, 1 per cent.	Degree of hemolysis.
	C. c.	*C. c.*	*C. c.*	*C. c.*	
1	[b]0.06	0.2	1.5	0.5	Complete.
2	.06	.15	1.5	.5	Do.
3	.06	.10	1.5	.5	Do.
4	.06	.05	1.5	.5	Partial.
5	.06	.03	1.5	.5	None.
6		.3	1.5	.5	None; control for hemolysin.

The titre of hemolysin according to the foregoing table would be 0.1, but the dose for each tube would be 0.25 c. c. A 1 per cent dilu-

[a] For the principles involved in this test the reader is referred to Bureau of Animal Industry Bulletin 136.

[b] This represents the dose of a titred complement. Wall uses instead 0.1 c. c. of 1:4 complement in his hemolysin titration scheme.

tion of red cells, which is recommended by Wall and used also by Holth and Larson, has been employed in this laboratory, although often a 2 and at times also a 5 per cent dilution have been used. The red cells both in concentrated as well as in diluted form, protected from light, keep well for 5 or 6 days at 4° to 6° C.

The complement, the third ingredient of the hemolytic system, is obtained as in the test for glanders by bleeding a guinea pig into a centrifuge tube by severing both the carotid artery and the jugular vein, after the animal has been stunned by a blow on the head. The blood is either allowed to stand over night in the ice chest for the separation of the serum or the blood after clotting is loosened from the sides of the tube and then centrifugalized and the serum pipetted off and diluted in the proportion of 1 to 4 of salt solution. The complement, although a nonstable substance, does not depreciate appreciably within 24 or 30 hours. The complement should, however, be titrated on the day the test is made. The titration is outlined in Table 3.

TABLE 3.—*Titration of complement.*

Tube.	Guinea-pig serum (complement), diluted 1:4.	Salt solution, 0.9 per cent.	Sensitized red corpuscles, sheep, 1 per cent.	Degree of hemolysis.	Remarks.
	C. c.	*C. c.*	*C. c.*		
1	0.08	1.5	0.5	Complete	
2	.06	1.5	.5	do	
3	.04	1.5	.5	do	
4	.03	1.5	.5	Almost complete	
5	.02	1.5	.5	Partial	
6	.01	1.5	.5	None	
7		1.5	.5	do	Control on sensitized corpuscles.
8[1]	.08	1.5	[2].5	do	Control on complement.

[1] This control can be omitted, but since we always have on hand nonsensitized washed red cells of sheep we include it in the test.

[2] Nonsensitized.

The ingredients are added in the same order as given in the table, then incubated at 37.5° C. for 1 hour, and then the evaluation of the complement is read. The titre according to the table would be 0.04 c. c., but in the final test 0.06 c. c. (the next largest quantity) would be used to each tube. This allowance was found necessary in order to produce complete hemolysis in our antigen controls (Table 5). The normal serum of cattle enhances the action of the complement in this hemolytic system. This was proven on several occasions when two series of complement titrations were executed, one series with and the other without the presence of normal inactivated cattle serum. The tubes of the series which contained the inactivated cow

serum invariably gave higher degrees of hemolysis than tubes containing the same amount of complement but no cattle serum.

The final tests.—The antigen used in the final test as prepared by the majority of workers is a serum bouillon culture of *Bacillus abortus* as recommended by Wall, which is preserved by adding 10 c. c. of 5 per cent phenol solution (or 10 c. c. of a solution composed of 5 parts of phenol, 10 parts of glycerin, and 85 parts of salt solution) to each 90 c. c. of such culture. Holth has used with equal success a filtrate of serum bouillon culture, suspension of bacteria in salt solution, alcoholic precipitate of filtrate dissolved in salt solution, and magnesium sulphate precipitation of filtrate. The writers have used glycerin bouillon culture 4 to 5 weeks old carbolized to 0.5 per cent, agglutination fluid, and the alcoholic precipitate of filtrate of Holth. While all three have given good results, the first is readily prepared and therefore most desirable.

The titration of the antigen is executed according to Table 4.

TABLE 4.—*Titration of antigen.*

Tube.	Antigen.	Inactivated serum of infected cow.	Complement[1] and salt solution, 0.9 per cent.	Incubation.	Sensitized red corpuscles of sheep, 1 per cent.	Incubation.	Degree of hemolysis.
	C. c	*C. c.*	*C. c.*		*C. c.*		
1	0.5	0.02	1.5	Placed in incubator at 37.5° C. for 1 hour.	0.5	Placed in incubator at 37.5° C. for 2 hours.	None.
2	.3	.02	1.5		.5		Do.
3	.2	.02	1.5		.5		Do.
4	.1	.02	1.5		.5		Do.
5	.05	.02	1.5		.5		Do.
6	.02	.02	1.5		.5		Partial
7	.01	.02	1.5		.5		Complete.
8[2]	.....	.02	1.5		.5		Do.

[1] The complement for this titration should be titred and the same dose as would be used in the final test should be used here. In order to facilitate matters the complement is added to the salt solution, so that each tube should receive the desired amount of complement plus the salt solution, the dose being made equal to 1.5 c. c.

[2] Tube 8 is a control on the serum.

Besides establishing the fixing quantity of antigen in the presence of a specific antibody, it is also desirable to establish the largest amount of antigen that may be used without inhibiting hemolysis, and for this purpose a similar series of tubes with ingredients as above described is used, differing only in using a serum from a non-infected animal and twice the dose of antigen in each respective tube. The fixing titre of the antigen in the presence of specific antibodies according to the above titration is 0.05, and the dose to be used in each tube may be made 0.15 or 0.2; provided, however, that the series executed with normal serum shows that twice that dose, that is, neither 0.3 c. c. nor 0.4 c. c. of antigen, will inhibit hemolysis. In the work

with the hemolytic system as used by us it was always necessary to make an allowance for the fact that the serum of normal cattle enhances the hemolysis. In fact, of late we have established the amount of antigen that will not inhibit hemolysis in a series similar to Table 4, but with double the amounts of antigen and the omission of serum in all but the last tube.

TABLE 5.—*Final complement-fixation test and titration of suspected serum.*

Tube.	Inactivated suspected serum.	Antigen.	Complement and salt solution.	Incubation.	Sensitized red cells, 1 per cent.	Incubation.	Degree of hemolysis.
	C. c.	*C. c.*	*C. c.*		*C. c.*		
1	0.1	0.15	1.5	Placed in incubator at 37.5° C. for 1 hour.	0.5	Placed in incubator at 37.5° C. for 1 or 2 hours.	None.
2	.05	.15	1.5		.5		Do.
3	.02	.15	1.5		.5		Do.
4	.01	.15	1.5		.5		Do.
5	.005	.15	1.5		.5		Do.
6	.002	.15	1.5		.5		Partial.
7	.001	.15	1.5		.5		Complete.
8	.....	.15	1.5		.5		Do.
9	.....	.3	1.5		.5		Do.
10	.....	.....	1.5		.5		Do.

Table 5 represents the final test in complement fixation and also the titration of a suspected serum. After the tubes are placed in the incubator for the second time they are allowed to remain there for from one to two hours, when results are read. The complete settling down of the corpuscles in tubes when hemolysis is not complete or altogether absent, will not be observed for several hours. In ordinary routine test tubes 1, 5, 6, and 7 are omitted and in their stead one tube containing the same as tube No. 2 but no antigen is used, which acts as a serum control, and this serum control must in all instances show complete hemolysis. Tube No. 8 is a control on the antigen dose. Tube No. 9 is a control on twice the antigen dose; No. 10 is a control on the hemolytic system. A known positive and a known negative is included whenever the test is applied to one or more suspected sera. This is a desirable step especially if all sera tested on one occasion give either positive or negative reactions and inasmuch as the antigen is titrated but once in every 4 or 5 weeks.

Interpretation of results.—1. Complete fixation of complement with serum in quantities of 0.02 c. c. or less should be considered as positive.

2. Partial fixation in 0.02 c. c. and complete in 0.05 c. c. should be considered as very suspicious and the agglutination test should be applied to the serum. If such serum shows agglutination in tubes containing 0.01 c. c. or less it may be considered positive. Should

it fail to agglutinate in quantities of 0.01 c. c. or less, another sample should be taken at the end of 2 weeks.

3. Sera failing to show a fixation titre of 0.05 c. c. quantity but giving an agglutination in 0.005 c. c. or in smaller quantities should be considered positive.

In over 400 cases the agglutination test failed to show positive results in only one case where a positive reaction was ascertained by the complement-fixation test, while conversely the latter test failed in four cases which were positive to agglutination, indicating in the latter tests that the agglutinins probably appear earlier in the infection than do the bacteriolytic antibodies. In view of these results it seems from our experience that the agglutination test alone, which can be carried out very readily with inexpensive apparatus and requires no great amount of time owing to the simplicity of the technic, would appeal to all interested parties, and only in doubtful cases would it be necessary to refer to the more complex complement-fixation test. A positive reaction to complement-fixation and agglutination tests does not prove whether the animal has aborted or is going to abort, but it does indicate that the animal is at present or has been infected with *Bacillus abortus*. In a general way it may be stated that in herds where infectious abortion is unknown no positive reaction has thus far been obtained.

In a few instances a positive reaction was not obtained in one or two aborting cows upon infected premises. In one case when negative results were obtained the exudate, fetal membranes, and discharge collected by one of the writers failed to reveal the presence of *B. abortus* after a careful search both microscopically and bacteriologically, suggesting that not all abortions are induced by the presence of *B. abortus*. In a herd of 25 cows where abortion had never been known to the best of the owner's knowledge, but where new stock had been added during the past year, 6 animals both new and old aborted, and the sera from every one gave high fixing and agglutinating titres.

The blood from 20 steers examined for the purpose of establishing the agglutinating power of normal cattle serum failed to show a single case where such serum had an agglutinating value of 0.01.

With few exceptions a positive or negative reaction when obtained will be observed with both tests. The agglutinating titre has been observed in many cases to be 10 times that of the fixing titre; thus a positive serum possessing a fixing titre of 0.02 will frequently possess an agglutinating titre of 0.002.

OTHER BIOLOGICAL TESTS.

Holth recommends the inoculation of the exudate from an aborting cow into a rabbit and that at the end of 7 days there will be a per-

ceptible increase of agglutinins and bacteriolytic amboceptors in positive cases.

ALLERGIC REACTION.

McFadyean and Stockman recommend for trial "abortin" as a diagnostic agent. "Abortin" is a material analogous to tuberculin and when injected subcutaneously or intravenously in 5 to 10 c. c. doses should give a rise of temperature to 104° F. or over within a few hours after the injection, in positive cases. The number of cases tested by McFadyean and Stockman were too meager to establish any definite conclusions, and they state that "it will be necessary to carry out a large number of tests in practice before deciding upon this method of diagnosis."

De Vine,[5] assisted by Malcolm, put "abortin" prepared by McFadyean and Stockman to a practical test with rather unsatisfactory results. Zwick did not find this agent reliable. Nonreacting animals would abort, and no reaction would be obtained in aborting animals. He also found disagreements between the "abortin" reaction on the one hand and the agglutination test on the other hand. We have prepared an "abortin" which we injected into cattle at the Bureau Experiment Station, and from the results obtained with our product it could not be considered nearly as reliable as either the agglutination or the complement-fixation test.

PREVENTION AND TREATMENT.

The principal method of treating infectious abortion is through prevention. No medicinal treatment has thus far been discovered for the cure of this disease, and the best methods of disinfection known to science are required to eradicate it from a herd. These procedures should be executed with the most exacting care and should include the disinfection of the animals as well as their surroundings.

When the disease has made its appearance in a stable the healthy cows should be changed preferably to an uninfected stable or premises. This is frequently difficult to carry out, and where it is not possible the aborting cows should be kept by themselves in another stable, or in an isolated portion of the stable with a temporary partition separating them from the healthy animals. Separate attendants should be provided for each herd, and there should be no communication of any kind between the two herds. If a cow develops prodromal symptoms of abortion she should be removed at once to the infected stable.

As soon as an animal has aborted, the fetus and membranes should be immediately carried away and destroyed by either burning or deep burial after covering with lime, as the abortion bacilli are extremely

numerous in these tissues. The vaginal discharge which follows is likewise very virulent and therefore should be disinfected, while the genital passages of the cow should be irrigated with an antiseptic solution and the animal kept from coming in contact with healthy cattle. The afterbirth, which is retained in most abortions occurring during the later months of pregnancy, should be removed within a few days. If it does not come away readily, do not forcibly remove it, but irrigate the uterus with a gallon or two of a warm disinfectant solution twice daily. This irrigation of the genitals is best accomplished by means of a soft rubber tube introduced into the vagina, and if possible into the uterus, with a funnel in its outer elevated end. About 1 gallon or more of a one-half to one-quarter per cent solution of liquor cresolis compositus, lysol, or trikresol, 1 per cent solution of creolin or carbolic acid, or 1 to 1,000 potassium permanganate solution, should be introduced into the womb, and this treatment should be repeated every day so long as any discharge is observed from the cow. Afterwards it should be used once weekly until it is time to breed the animal. In addition this cow, as well as every cow in the stable, should be sponged every morning around the vulva, anus, perineum, and root of the tail with a disinfectant solution twice as strong as that used for irrigating the genitals. Furthermore, every cow in an infected herd should have the genital tract irrigated as above, even after an apparently normal parturition. W. L. Williams reports very good results from using one-fourth to one-half per cent Lugol's solution for irrigating the vagina during one estrual interval—that is, a period of 21 days—before breeding. The use of this solution is said not to prevent conception even if used one hour before service.

It is not advisable to breed a cow for at least two months after she has aborted, and not even then if the discharge has not ceased. If these precautions are neglected and the bull is allowed to serve the cow as soon as she comes in heat after aborting, the uterus will not be normal, and the animal will not conceive or the fetus will be expelled when quite small, while in a short time the cow comes in heat again. These very early abortions are as a rule not noticed, but as the system of the cow adapts itself to the infection, either through tolerance, immunity, or a loss of virulence of the bacilli, the period of retention becomes longer and longer, until finally the cow is immune and carries the fetus the full term of gestation. It generally requires from two to three years for the cow to become immune and even then there is a possibility of the cow acting as a carrier of the virus, and the bull which during that time serves this cow may transmit the infection to all other cows that he may cover if precautions are not taken to prevent it. For this reason it is not

advisable to sell or otherwise dispose of the animals that abort and replace them with new cows, as such new animals are very likely to become infected. (See Table 6.) Only those which after treatment prove to be permanently sterile should be prepared for the butcher.

In order to prevent a bull from carrying the infection from a diseased to a healthy cow, it is necessary to irrigate and disinfect the sheath and penis before and after each service. Following the clipping of the long tuft of hair from the opening of the sheath, the end of a small rubber hose is inserted into the sheath and the foreskin held together with the hand to prevent the fluid from flowing out again immediately. The other end of the hose contains the funnel, into which any of the above-mentioned antiseptics used in irrigating the vagina is poured, and the prepuce sack is flushed out. The injection may also be made by means of a common fountain syringe with a long nozzle. The skin of the abdomen around the sheath should likewise be sponged with a disinfectant.

When a stable has become infected, it should be carefully and thoroughly disinfected. The cattle should be removed and the stable kept empty for two or more days. The walls, floors, and gutters should be scrubbed and the ceiling brushed clean of dust and cobwebs, and then a 3 per cent solution of liquor cresolis compositus, lysol, carbolic acid, etc., should be applied with a force or spray pump so as to force the disinfectant into the cracks and crevices. This disinfection should be repeated after each abortion. In addition to the above measures it is necessary to clean out the barnyard, removing the manure and contaminated litter to some field not accessible to cattle, where it is plowed under. The surface of the yard should be sprinkled with a solution of copper sulphate, 5 ounces to a gallon of water. Milking stools and other implements should also be thoroughly disinfected.

Great care should be taken to guard against cows or bulls from another aborting herd, and workmen who have attended such a herd should be made to wash and disinfect their clothes and persons before going into a healthy herd. The purchase of infected cattle may at the present time be prevented by demanding that such animals shall come from a herd, the members of which show a negative reaction to the complement-fixation and agglutination test for infectious abortion. Otherwise, all newly purchased cows should be kept separate from the healthy herd until they have calved.

It is not to be expected that this disease can be suppressed at once, but by keeping up the above treatment the losses will be diminished and the disease finally eradicated.

With reference to medicinal treatment, various agents have been recommended and heralded as specifics from time to time, but the beneficial results attending their use may be attributed more to the

nature of the disease or errors of diagnosis than to the therapeutic action of the drugs. In some cases similar to those cited in the chapter on Symptoms it appears that the cows are preparing to abort, and if any drug should be used at this time, credit would probably be given the remedy as the cause of the continuation of pregnancy, whereas such symptoms may abate without medication. Carbolic acid has been the most widely recommended agent in the treatment of this disease, and good results have been reported by subcutaneous injections of 2 drams of a 2 per cent solution every week until 12 injections have been made. The most suitable place for the injection is on the side of the neck. Range cattle may be more readily treated by the use of medicated salt placed in troughs accessible to the cattle. This salt may be prepared by pouring 4 ounces of liquefied crude carbolic acid upon 12 quarts of ordinary barrel salt and mixing thoroughly. The reported success of this carbolic acid treatment is probably more the result of the tolerance or immunity to the disease which occurs after several abortions rather than the effect of the remedy itself.

IMMUNIZATION.

A careful examination of various herdbooks will show that cattle rarely abort more than two or three times, after which they develop a tolerance or resistance to the infection and carry the fetus to the normal termination of pregnancy. In this manner the disease will gradually exhaust itself after several years, providing susceptible animals are not purchased and added to the herd. It is this tendency toward natural immunity of the infected cattle which has raised the question of the production of an artificial immunity by various methods of procedure. Bang's investigations along this line indicate the possibility of securing such immunization in cattle as well as sheep and goats. He worked with both living and dead cultures of the causative bacillus and injected them intravenously and subcutaneously. McFadyean and Stockman have also experimented with the same purpose in view and have obtained encouraging results from the use of large doses of a virulent culture subcutaneously six to eight weeks before breeding.

The writers have been conducting a number of experiments on five large dairy herds in an endeavor to find a suitable biologic product for immunization and control of this disease, and to this end suspensions of abortion bacilli killed by heat or carbolic acid (0.5 per cent), or those no longer viable on account of age, were injected as above mentioned as well as intraperitoneally and by ingestion. Some of the experiments were performed on animals at various intervals before breeding and in other cases both before and after breeding. It should

be remembered that complete immunity by natural infection is slow in development, for whereas one active siege of the disease, as manifested by an abortion or possibly by a retained afterbirth, produces immunity in many instances, nevertheless in a considerable number of cases immunity occurs only after a prolonged siege or reinfection, as manifested by two or more abortions in the same animal. (See Table 6.) In view of these facts the writers have further extended their efforts toward fortifying the infected or exposed animals during pregnancy by the use of dead or avirulent bacteria, thereby stimulating not only the production of bacteriologic and other antibodies, but also the increase of the opsonic index. Over 250 head of cattle have been thus treated from three to five times, but only a very small proportion of this number have as yet calved. In a small herd treated early in this work the results were not encouraging, but failure in those cases may be attributed to the facts that a very thin suspension of bacterin had been injected and, secondly, just one strain had been used in the preparation of the product. Since the different strains have been found to vary somewhat, subsequent experiments have been conducted with denser suspensions made from a number of the most virulent strains, and the preliminary results thus far obtained with these injected cattle have been more satisfactory. For a more definite decision on the value of this line of vaccination the results of the treatment of the latter animals must be awaited, but the outlook for prophylactic treatment along this line is somewhat encouraging and will furnish a basis of a separate report as soon as the investigation is completed. Studies are also being conducted with reference to the immunizing effect which infected milk from a "bacillus carrier" mother will have upon the calf when it becomes adult.

Table 6, on the next page, gives the histories of certain cows in a herd affected with infectious abortion, showing a number of cases in which tolerance or natural immunity has been acquired.

We desire to express our indebtedness to Dr. J. P. Turner, veterinarian in the health department of the District of Columbia, for various courtesies extended in connection with the study of infectious abortion, and especially for the information contained in this table. Dr. Turner states that several cows which recently aborted but which have not as yet been bred are not mentioned in the table. A number of the cows mentioned that have either aborted or gone the full period in 1912 are again with calf.

TABLE 6.—*Records of aborting cows in a herd affected with infectious abortion.*

No.	Date of introduction into herd.	Age at that time.	Pregnancies in 1908.	Pregnancies in 1909.	Pregnancies in 1910.	Pregnancies in 1911.	Pregnancies in 1912.	Disposition of animal.
		Years.						
6	June 26, 1907	7	Full period	Aborted, 5 months	Full period	Full period	Full period	Still in herd.
22	1908	10	do	Aborted, 7 months	do	Aborted, 8 months		Sold, 1912; poor milker.
28	1908	6	Aborted, 7½ months	Full period				Sold, 1910; poor milker.
37	1908	8	Full period	do	Full period	Aborted, 7 months		Sold, 1912; poor milker.
57	1908	12	do	do	Aborted, 6 months			Sold, 1910; poor milker.
62	1908	8	Aborted, 8 months					Sold, 1909; poor milker.
66	Jan. 27, 1908	6		Full period	Aborted, 7 months	Full period	Full period	Still in herd.
80	do			do	Aborted, 6 months			Sold, 1911; poor milker and failed to breed.
93	1908	13	Full period	do	Aborted, 5 months			Sold, 1910; poor milker.
99	Jan. 18, 1907	9	do	Aborted, 7 months				Sold, 1909; poor milker.
100	1908	12	do	8 months				Sold, 1910; poor milker.
133	1908	11	do	Full period	Full period	Full period	Aborted, 8 months	Still in herd.
136	1908	6	do	do	Aborted, 7 months			Sold, 1910; poor milker.
147	June 20, 1908	5		do	do	Full period	Full period	Still in herd.
155	June 27, 1908	2	Full period	do	Aborted, about 6 months.	do	do	Do.
158	June 2, 1908	6		Aborted, 7 months	Full period	do		Sold, 1912; poor milker.
167	July 10, 1908	5		Full period	Aborted, 8 months	do	Now past eighth month.	Still in herd.
170	do	5		Aborted; stage not known.		do	Full period	Do.
171	do	5		Aborted, 6 months	Full period	do		Sodl, 1912; unprofitable.
173	do	4		Full period	Full period	Aborted, 5 months	Now in fourth month.	Do.
175	do	6		Aborted, 4 months	Aborted, 5 months	Full period		Do.
178	July, 1908	6	Full period	Aborted, 6½ months				Sold, 1910; poor milker.
181	Nov. 20, 1909	7		Full period	Aborted, 7 months			Sold, 1911; poor milker.
182	do	4			Aborted, 5 months	Full period	Full period	Still in herd.

187	May 9, 1909	6	.....	Full period.....	Full period.....	Aborted, 7 months..	.....	Sold, 1912; poor milker.
190	.....do.....	6	.....	.....	.....do.....	Aborted, 6 months..	.....	Sold; failed to breed and poor milker.
192	.....do.....	5	.....	Full period.....	.....do.....	Aborted, 7 months..	Full period.....	Still in herd.
194	.....do.....	6	.....	.....do.....	Aborted, 7½ months...	Full period.....	.....do.....	Do.
195	May 21, 1909	6	.....	.....	Aborted, 8 months....	.....	.....	Sold, 1910; poor milker.
199	May 22, 1909	3	.....	.....	Aborted, 6 months....	.....	.....	Do.
202	May 30, 1909	5	.....	.....	Full period.....	Aborted, 5 months..	.....	Sold; poor milker.
203	.....do.....	6	.....	.....	Aborted, 8 months....	Full period.....	Full period.....	Still in herd.
207	June 16, 1909	4	.....	.....	Aborted, 6 months....	.....	.....	Died, 1910.
209	.....do.....	.....	.....	.....	Aborted, 8 months....	.....	.....	Sold; poor milker.
218	June 26, 1910	5	.....	.....	.....	Aborted, 4 months..	Full period.....	
221	.....do.....	6	.....	.....	.....	Full period.....	Aborted, 6 months..	Sold, 1912; poor milker.
222	.....do.....	5	.....	.....	.....	Aborted, 6 months..	Full period.....	Still in herd.
223	.....do.....	5	.....	.....	.....	.....do.....	.....do.....	Do.
227	Dec. 2, 1907[1]	.....	.....	Aborted; pasture bred.	.....	Full period.....	.....do.....	Do.
239	July —, 1908[1]	.....	.....	.....	Aborted; pasture bred.	.....do.....	.....	Sold, 1912; poor milker.
242	Sept. 5, 1908[1]	.....	.....	.....	Full period.....	Aborted, 7 months..	.....	Sold; poor milker.
250	Nov. 29, 1908[1]	.....	.....	.....	Aborted; pasture bred.	.....	Full period.....	Still in herd.
253	Dec. 7, 1908[1]	.....	.....	.....	.....do.....	.....	.....do.....	
254	Jan. 14, 1909[1]	.....	.....	.....	.....	Full period.....	Aborted, 6 months..	Still in herd.
258	Mar. 15, 1909[1]	.....	.....	.....	.....	Full period; then aborted at 5 months.	Aborted, 5 months..	Do.
262	May 2, 1909[1]	.....	.....	.....	.....	.....	Aborted; pasture bred.	Do.
297	Sept. 21, 1910	7	.....	.....	.....	Aborted, 7 months..	Full period.....	Do.
320	June 26, 1910	.....	.....	.....	.....	.....	Aborted, 8 months..	Do.
323	.....do.....	.....	.....	.....	Full period.....	Aborted, 7 months..	Full period.....	Do.
326	.....do.....	.....	.....	.....	.....	Full period.....	Aborted, almost 9 months.	Do.
352	Nov. 21, 1910	4	.....	.....	Full period.....	Aborted, 6 months..	Aborted, 7 months..	Do.

[1] Date of birth. An examination of this herdbook further shows that only one aborting heifer (239) was produced by an aborting mother (178). With this exception all the aborting cows gave birth to heifers which have records of from 1 to 3 successful pregnancies without any abortions.

MEAT INSPECTION.

From the standpoint of meat inspection it is impossible to tell without any history whether an animal is or is not affected with infectious abortion merely from an ante-mortem or post-mortem examination by the inspector. The causes for condemnation in these cases would be such complications as sapremia, puerperal septicemia, metritis, endo-metritis, retained placenta, peritonitis, pyometra, mummification of the fetus, and other similar conditions which may be observed in this disease.

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